CHEMISTRY IN MEDICINE

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CHEMISTRY IN MEDICINE

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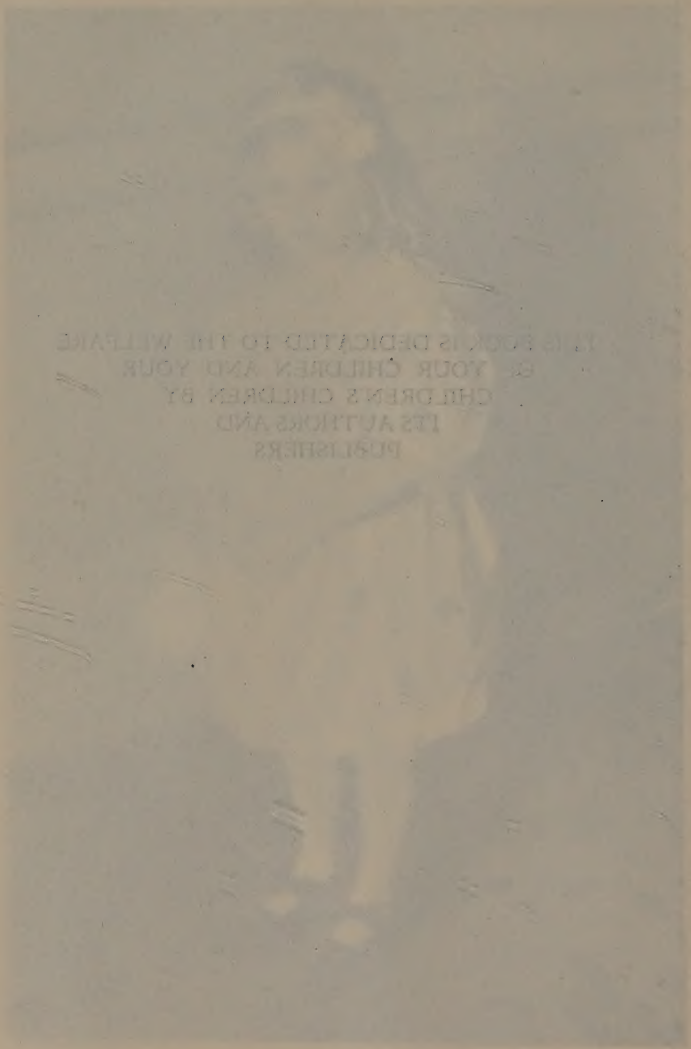
MAY THE MEMORY OF LOST CHILDREN URGE US ON

THIS BOOK IS DEDICATED TO THE WELFARE
OF YOUR CHILDREN AND YOUR
CHILDREN'S CHILDREN BY
ITS AUTHORS AND
PUBLISHERS



CHEMISTRY IN MEDICINE

A COOPERATIVE TREATISE INTENDED TO GIVE EXAMPLES OF PROGRESS MADE IN MEDICINE WITH THE AID OF CHEMISTRY

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FOREWORD

THIS book is intended to present to the public as well as to the practitioners of medicine the great possibilities for advance in medical science through further intensive cooperation between chemistry and medicine. The impetus which has led to its preparation had its origin some years ago in the tragic loss of a beloved child, Patricia, by Mr. and Mrs. Francis P. Garvan of New York City. Happy and in the best of health, the delight of her parents and of their friends, Patricia was stricken by rheumatic fever, following grippe, before which some of the best of the country's physicians, drawn into consultation, confessed themselves utterly powerless. Within a week the bright little life was lost. But the gracious spirit of the child left in the minds of the parents the conviction that if medicine had failed them, it had failed in spite of the devotion of its practitioners and only because of the vast gaps in our knowledge of the ultimate causes of many diseases and of scientific methods to combat them. Further thought and conferences with scientific friends led them to the conclusion that one of the most promising methods of insuring progress in the attack on some of the great unsolved problems of medicine is to be found in cooperation of medicine with chemistry. Chemistry is the fundamental science of the transformation of matter, and our life, from our inception to our return to dust, in

every breath we draw and in every moment we live, is indissolubly connected with most complex transformations of matter. These take place in our blood, in our tissues and our organs. Admittedly the life sciences still know far too little about these complex but fundamental chemical phenomena to make possible that clear insight into and positive control of factors of life and health, which must be the basis of a science of medicine. For over ten years Mr. and Mrs. Garvan have devoted their thought, strength and means to the upbuilding of American Chemistry:

First—To aid in establishing independent and self-sufficient American Chemical Industries.

Second—To establish in our country a better understanding of the possibilities and purposes of chemistry.

Third—To foster School, College and Postgraduate Education in Chemistry and to advance, through the agency of scientific societies and universities, the cause of Chemical Literature and Chemical Laboratories.

Fourth—To advance the cause of adequate American Chemical Research facilities.

These have appeared as essential to their goal—American leadership in the application of Chemistry to the advancement of our scale of living, to a wider scope of knowledge and now particularly to the advancement of the safety and health of the world's children and its children's children.

For the further advancement of the cause of cooperative effort of medicine and chemistry, the present volume was undertaken at the request of Mr. and Mrs. Garvan, and it is published without profit by The Chemical

Foundation, of which Mr. Garvan is president. It is dedicated to the protection of children and adults against avoidable disease and death.

Past achievements form the most dependable test of the possibilities inherent in cooperation between chemistry and medicine. In the chapters that follow, the story will be told of some of the most important successes, already assured, in the combating of disease and alleviation of suffering through this alliance of sciences. It should be well understood that the part of chemistry is played under many names: we have not only the chemist himself, but also the biochemist and the physiologist applying chemistry to the study of life phenomena; the pharmacologist investigating the effects of drugs on the body; the bacteriologist in many of his operations relies upon chemistry and in turn adds vitally to the contributions of chemistry; many leading medical investigators have systematically used chemistry as their tool to achieve their results. It is in this broad sense that the rôle of chemistry has been outlined. The authors include eminent physicians, pathologists, pharmacologists, physiologists, bacteriologists as well as chemists. It should also be understood that in many important instances chemical and non-chemical medical methods are so closely interwoven in achieving progress that no effort is made to discriminate between the sciences: cooperation is the keynote and progress the objective, however it may be attained.

Each story will be told by a specialist in his field and as far as possible in non-technical language that any layman can follow. The epic which these stories unfold must bring home to all a feeling of grateful wonder at

what has already been accomplished. But through the whole story, the observant reader must be impressed by the vast gaps which still exist in our knowledge, gaps which the authors themselves emphasize. We have made a little, but only a little, progress in the prevention and treatment of pneumonia, tuberculosis, cancer, high blood pressure and its relations to the heart, the kidneys and the circulation in the brain, diseases to which the greatest proportion of man falls victim. Our children are still exposed to infections which can wipe out their precious lives in a few days or which may leave them crippled with heart disease, infections before which even the wisest of physicians stand helpless. Even some of our greatest triumphs have not yet been made altogether safe: The child that has been saved from an attack of diphtheria by diphtheria antitoxin may be sensitized to horse serum, its vehicle; should a common accident of childhood, a torn hand or leg require protective antitetanus treatment for the same child, he may stand in positive danger of death by anaphylactic shock from the vehicle, horse serum. The preparation, in recent years, of antitoxins with the aid of sheep as well as horses, is a valuable aid in reducing these dangers, but it does not solve the fundamental problem of furnishing medicine with the pure antitoxins from which the dangers of serum sickness have been entirely removed. While chemists are working ardently on this problem, progress is all too slow in the face of the thousands of children whose safety is involved.

The transmission of good health as well as good character to our offspring is of transcendental import-

ance. For this reason we are starting the series of treatises with a chapter on heredity and development. It is replete with brilliant successes of the purely biological science of genetics. The contributions of chemistry thus far are almost vanishingly small. And still, biologists are convinced that final answers to these problems can only be found in the chemistry of life. We know already that each species has its own specific proteins, the injection of which into a sensitized animal of another species, may cause the most violent reaction and even death. The chapter on heredity and development stands as an invitation for intensive cooperation of the fundamental sciences of chemistry and physics with the biological sciences in the most momentous of all problems in the protection of health and happiness.

Here are indeed vital problems for the solution of which mankind waits. Let us realize that every year gained in the making of a fundamental discovery in medicine means the saving of tens of thousands of lives and the warding off of unhappiness from many thousands of homes. Five years ago diabetes in childhood was almost invariably fatal; today insulin is preserving the lives of thousands of happy little children. Can one realize the feelings of stricken parents whose children were taken from them just before the dawn of this new day? The most effective way to accelerate the successful issue of any undertaking is to attract to its standards the brainiest and boldest among men and, in this case, to multiply the number of well trained investigators.

Practically, this can be accomplished by more generous support of research institutes and of research in univer-

sities and by more intensive participation of the government in fundamental research as distinguished from work of control. In all three of these active centers of research more generous financial support would not only attract a larger proportion of ambitious and able men and would not only multiply workers, but would enhance productiveness by removing the depressing influences of financial worry and the need of enforced outside activity to replenish income. This latter is the greatest handicap American research is carrying. It is hoped that this volume may help to arouse the public to a clear understanding of these simple facts.

It is due to our readers to say that all of the authors of our chapters, as well as all of the editors, have contributed their services gratuitously to this humane undertaking. The editors take this opportunity to thank all of the contributors for their very gracious cooperation. Distinguished each in his own field and busy as professional men and women are busy, they nevertheless have taken time and thought to prepare these articles because their hearts are in the cause. Any life saved, any suffering ameliorated, any future discovery hastened by reason of this volume will more than compensate them for their efforts.

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CHAPTER I

The Significance of Chemistry and of Its Methods of Attack on Fundamental Problems

JULIUS STIEGLITZ, PH.D.

Professor of Chemistry, University of Chicago

Dr. Stieglitz's connection with the University of Chicago dates from 1892; he has been Professor of Chemistry since 1905, and chairman of the department since 1915. During the war he was chairman of the sub-committee on synthetic drugs of the National Research Council and since 1918 he has been a special expert of the U. S. Public Health Service. From 1905 to 1919 he served on the Council on Pharmacy and Chemistry of the American Medical Association and in 1918 was President of the Institute of Medicine of Chicago. He is one of the co-authors of the report on "The Future Independence and Progress of American Medicine in the Age of Chemistry" (1921) and in 1924 he gave the Dohme Lectures on "Chemistry and Recent Progress in Medicine." He is a member of the National Academy of Sciences.

SINCE the emphasis in this book is to be placed on the services of chemistry to medicine, it may be of interest to some readers to consider first why chemistry should be so deeply involved in life phenomena as to become of necessity a partner with medicine in its effort to preserve life. It will then be of further interest to consider somewhat in detail the general methods of attack of chemistry on the problems it tries to solve. It is these methods which have made modern chemistry a powerful and resourceful machine. The confidence of the reader in a final successful solution of

many difficulties facing medicine will be the greater the more clearly he understands the processes of thought and experimentation by which chemistry handles its problems.

Chemistry is the science whose particular field is the study of the transformation of matter. Transformations of matter underlie thousands of processes and products of the utmost concern to man. Comparatively simple transformations are those by which useful metals, such as steel, lead, copper, and many others, are prepared from crude ores. All of these processes are chemical in character and they have been brought to their present state of high efficiency under the supervision of chemists. It is the chemist who has placed at the disposal of man steel of every conceivable quality by the clever admixture with iron of such other ingredients as carbon, silicon, manganese, chromium, nickel, and other metals.

CHEMISTRY AND LIFE

This power to vary the qualities of a common product under the discriminating guidance of the chemist must make us realize the possibilities of the same power intelligently applied to life phenomena. For life in its every phase involves transformations of matter far more important to us and far more astounding, than the transformation of ores into useful metals. We sow our fields with grains of wheat or corn: in the warm, moist earth the tiny germs first transform the stored food, proteins, starches, fats, with which they are surrounded in the seeds, into rootlets to draw water, ammonia, nitrates and other mineral salts, from the soil for their further nourishment: the germs then send up stalks and leaves to capture with the aid of the sun carbon from

the carbon dioxide of the air. It is an old story now how the chemist by the addition of fertilizers to the soil, such as potash, phosphate, lime and ammonium nitrate, multiplies the yield of the soil and improves each individual plant.

But coming closer home to us and stirring us even more profoundly are the transformations that follow the fertilization of the female germ cell, which starts chemical processes that continue in almost infinite variety as long as the life of the individual lasts. With the aid of eggs of the sea-urchin, Jacques Loeb demonstrated in a series of famous experiments that the development of the egg could be started not only by the male sperm of the sea-urchin but by chemicals added to water bathing the eggs. Dr. Loeb was kind enough some years ago to show the writer his classical experiments at Pacific Grove: Under a microscope a slide showed one or two sea-urchin eggs as clear round globules; a drop full of wriggling sperm was added; almost instantly, the moment a spermatozoon penetrated into the egg, a membrane closed the cell, and cell division started so rapidly that within one or two minutes innumerable small divisions were seen where there had been a single clear cell. Then the experiment was repeated on another slide; a few drops of chemical solution, free of sperm, were added to a clear cell or two; the same changes followed, perhaps a trifle more slowly. The author recalls no more thrilling experience than this adventure with Jacques Loeb, which connected life at its threshold with chemical activity. Similar experiments have been made with the eggs of frogs, and the eggs, started on their way by sperm or chemicals, have been carried to the development of frogs through all of its interesting stages.

The fertilized human ovum, a single minute cell, grows by a multiplication of cells, which is made possible by the nourishment absorbed from the mother and transformed in the chemical laboratories represented by the cells. These grow to myriads in number, and, in health, they are wonderfully efficient. In the adult as in the child all life functions continue to be carried out by cell activities. The cells absorb and secrete chemicals such as water, sugars, salts, fats, amino acids and proteins; they transform these into the more complex substances required by the body; they make possible the oxidation of sugars, fats and proteins to furnish warmth and energy; and finally, they provide for the excretion of waste products. Fundamentally the problem of health is a problem of normal cell processes. And still, our understanding of the fundamental processes occurring in cells is so meager that a complete knowledge of the chemical components of even a single cell has never as yet been attained.

That progress of the greatest moment to the saving and prolonging of life has, nevertheless, been made through the application of chemistry to life problems will be amply demonstrated in the chapters that follow. Impressive as the results must be, it is well to understand even at the outset that the gaps in our knowledge are far greater than any successes attained. We still have millions of people sick and dying every year, who, with the advance of science, would have a longer lease of life and happiness.

METHODS OF CHEMISTRY

And now let us turn to the consideration of the most important of the methods which chemistry uses in its

attack on problems. For it is these methods which not only have enabled chemistry to render great service in the past, as outlined in the chapters that follow, but which are the basis of our complete confidence in further great contributions to the progress of medicine in the future.

Analysis.—Chemistry begins its work by taking apart, by analyzing every material whose transformation it wishes to understand and control. If it is a simple case, as an iron ore, the ore is first analyzed and every single component determined as to its quality and its quantity. If too little iron is found present to pay the cost of transformation into steel, the ore is rejected. If such ingredients as manganese or phosphorus or titanium are found in the ore, the process of making steel out of the ore is adjusted to their presence. It is not guess work—the chemist is guided by his analysis and he gives the steel manufacturer the opportunity to prepare economically a product of high quality.

In a similar fashion the extraordinarily complex mixtures represented by our bodies, in their tissues and fluids, are subject to the analyzing curiosity of the chemist. For the present, they are too complex for any complete analysis, but as the prospector looks for ore in mountain ranges, or for oil in plain and prairie, so the chemist searches for particularly precious ingredients in the materials of the body. As the need arises, he develops ingenious new methods of analysis to capture the elusive object of his search. Thus, Abel at Johns Hopkins University isolated the pure chemical principle or hormone epinephrine (often called adrenaline) secreted by capsules located on top of the kidneys. Epinephrine is a powerful heart stimulant and it contracts blood capillaries. As a

result of this analytical quest, medicine uses epinephrine now to allay the spasms of asthma, to stimulate the heart and occasionally to save the life of a stillborn babe, and for many other important purposes. Kendall of the Mayo Foundation sought out and isolated thyroxine from the thyroid gland; a glance at his pictures (p. 234) of a little girl, a dwarf in mind and body as a result of a deficiency of the normal thyroid secretion, before and after the treatment with minute doses of thyroxine, tells the story of the importance of this chemical principle. In a brilliant campaign of analysis, a model of cooperative research, Banting, Macleod, Collip and Best of the University of Toronto, discovered insulin, the principle in our blood which makes possible the oxidation and utilization of sugar—and the world is safer and life brighter for hundreds of thousands of diabetics. In the chapters that follow, instance after instance will be found of the penetrating power of chemical analysis and of the benefits that flow from it.

In his pursuit of knowledge by analysis, the chemist is not content to stop with the isolation of pure principles such as iron, epinephrine and other important products. The pure principles themselves, once isolated, are objects of his dissecting curiosity. He finds that epinephrine and thyroxine can be resolved into carbon, hydrogen, oxygen and nitrogen; insulin into these and sulphur as well. But iron cannot be resolved—it is a chemical element. Carbon, hydrogen, oxygen, nitrogen and sulphur are other chemical elements. Now, there are thousands of combinations of carbon, hydrogen, oxygen and nitrogen, with or without sulphur. What differentiates epinephrine with its peculiar powers from these other thousands of compounds of the same ele-

ments? To answer this and all other similar questions, the chemist is compelled to push his inquiries much further, to the very atoms of which the smallest particles (called molecules) of any pure principle are composed.

The Atomic Theory.—The great English chemist John Dalton was the first to demonstrate clearly that the chemical elements consist of atoms; that each element has its own kind of atom of a characteristic mass or weight,* and of specific chemical activity. The atomic theory has been an extraordinary aid to chemistry in its aim to penetrate to the most intimate knowledge of our world and through chemistry the theory has been of the greatest benefit to mankind. It has been so because it has carried the powers of analysis and the penetration of knowledge far beyond the limits of our most powerful microscopes. This will be understood if we consider that a single drop of water contains $2x10^{21}$ molecules of water, each molecule consisting of three atoms, two of hydrogen and one of oxygen, as expressed by the well known formula H_2O.** To grasp the meaning of this number $2x10^{21}$, consider that there are nearly 2,000,000,000 people on the face of the globe and that if each one of these were to count three molecules of water per second, it would take all of the inhabitants of the earth something over 10,000 years to count the molecules in one drop of water.

It will be worth our while to become somewhat more familiar with the atomic theory and its applications. Such a review will prepare the way for the ready under-

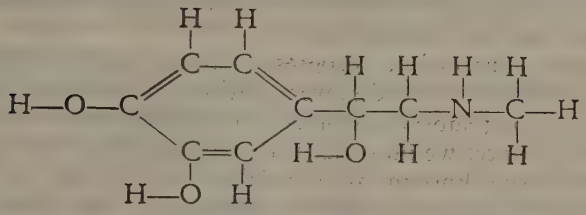
*Thus the carbon atom is 12 times, an oxygen atom 16 times as heavy as a hydrogen atom.

**H designates an atom of hydrogen, O one of oxygen, Cl an atom of chlorine, C one of carbon, N an atom of nitrogen, etc.

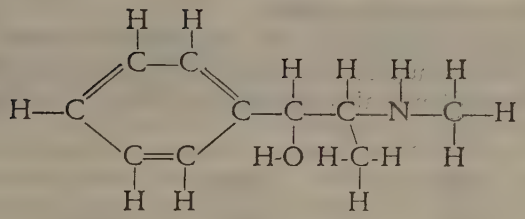
standing of some important parts in the chapters that follow. Each one of the great variety of material substances which we find in nature, in the stars, in rocks and minerals, in plants and animals, is an element or the product of some chemical combination of elements. The union of elements takes place through the combination of their atoms. This corresponds somewhat to the formation of the family as the unit of modern civilized communities. Often a single atom of an element will combine with a single atom of another element, as hydrogen combines with chlorine to form hydrochloric acid (HCl), the acid in the digesting fluid of the stomach; often other simple forms of combination occur, as expressed by H_2O for water, NH_3 for ammonia and CH_4 for marsh gas.

Molecular Structure.—But the most important of the materials which are concerned in the functions of life are far more complex. Thus, the smallest particle of epinephrine, its molecule, is composed of nine carbon atoms, thirteen hydrogen atoms, three oxygen atoms and one nitrogen atom, as expressed in the formula $C_9H_{13}O_3N$. Even this knowledge is not sufficient to shed light on the nature of epinephrine, as little as a bald statement of the tons of steel, brick and stone in any edifice would give us any adequate knowledge of the nature and functions of the edifice. One of the triumphs of chemistry is that it has acquired the art of determining how the atoms are linked together in molecules. Thus water is H-O-H and not H-H-O. The problem is more difficult for a complex molecule like epinephrine. By breaking it up and studying its fragments, chemists have arrived at the following as a satisfactory picture of the way in which the 26 atoms in the mole-

cule of epinephrine are linked together (called the "structure" of epinephrine):



Epinephrine



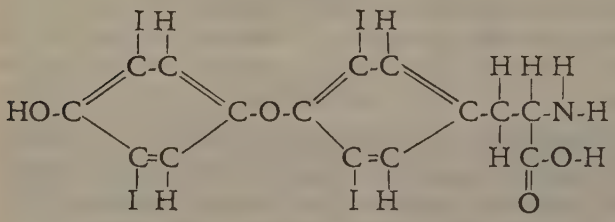
Ephedrine

By considering the groupings of atoms in the molecules of related substances, some knowledge is acquired of the connection between function and molecular structure. This has been carried furthest in the study of the dyes; all dyes have in common certain groupings of atoms in their molecules, which make color possible. Exactly by this kind of exhaustive investigation, chemistry has acquired its complete mastery of this field. It is now indeed possible to make at will dyes of any color, shade or tint; dyes of any degree of resistance to fading in the light and air or of resistance to the efforts of the

laundress; or dyes of such a degree of sensitiveness to color and light that photographic plates, colored with such dyes, now reveal clearly what fogs and distance hide from the ordinary plate.

Slow but definite progress is being made in the far more vital effort to discover relations in molecular structure among substances of definite importance to life. As one instance, we find that the effects of a new and powerful drug known as ephedrine* are similar in important respects to those of epinephrine; for some purposes ephedrine is a useful substitute for epinephrine. With this similarity in effect we note a remarkable similarity in structure (see above).

Even more important is the fact that the chemist can see in the structure of thyroxine, the active principle of the thyroid gland, also important points of similarity



to epinephrine. Progress in the isolation of other pure principles of secretion (hormones) will make possible the determination of their structures and this knowl-

*Ephedrine, it may be added, is the active principle of the Chinese drug ma huang, which has been used in China empirically for very many years; the pure principle was isolated by Nagai in 1887.

edge promises to lift the veil from many obscure fundamental biological relations. One such problem is found in the remarkable interrelations between the various hormone producing glands, which are well known to physicians.

When we consider that the conviction is growing that even the great problems of heredity are intimately connected with questions of this kind, we must appreciate the importance of this deep, searching analytical quest of chemistry into the very structures of the molecules which contribute to life's complex picture. Possibilities for power over life are as inviting here as they have been successful in the domain of dyes.

We shall presently see that chemistry is already making practical use of this knowledge of molecular structure in many instances. It does so for two important purposes, the one to prepare artificially these peculiar life products and life regulators (such as epinephrine); and the other object, to prepare, wherever it is possible. something better than nature offers—truly a worthy objective of man's mind and courage.

The Structure of Atoms.—But we are anticipating somewhat in our story. Let us first complete the tale of the analytical adventuring of chemistry by recalling that in our own generation the atoms themselves have been invaded and their structure studied by physicists and chemists. These investigations have led to the assumption that atoms are composed of two ultimate forms of matter, both electrical in character. The first of these is considered to consist of atoms of negative electricity which are called electrons. Their presence in the atoms of all elements was deduced by J. J. Thomson from his observations on the nature of cathode rays (in an X-ray

tube) in 1897 after a number of earlier investigators had found indications of their existence. An electric current flowing through a wire is supposed to be a current of swiftly moving electrons. The second ultimate form of matter is said to consist of positive atoms of electricity, usually called protons, but sometimes termed positive electrons. They were discovered by Rutherford in 1911. Thomson showed that the negative electron has a mass of 1/1850 of the mass of the hydrogen atom, and Rutherford demonstrated that the mass of the proton is that represented by all the rest of the hydrogen atom. In other words, a hydrogen atom is considered to consist of one rather massive proton which is supposed to form a nucleus in the center of the atom, and of one very light electron, moving in an orbit around the proton, somewhat like the moon around the earth. All atoms are regarded as constellations of protons and electrons. The number of these in the case of a given element corresponds to the atomic weight of the element: thus, the carbon atom C consists of 12 protons and 12 electrons, the oxygen atom O of 16 protons and 16 electrons.

If we bear in mind that positive electricity and negative electricity attract each other, we can understand that chemical combination is possible because of electrical attractions between atoms. Thus, two atoms of hydrogen $H^{+}\ominus$ are found to combine with each other to form a molecule of hydrogen H_2 with the electrical structure $H^{+}{}^{\ominus}_{\ominus}$ +H. The two negative electrons $\ominus$ are supposed to be moving in orbits between, or around, the two positive particles H^{+} and the forces of attraction between positive and negative electricity make the

molecule H_2 possible. The intensity of these attractive forces is made evident by the fact that from the union of atoms of hydrogen Langmuir has realized the hottest flame known, a temperature of at least 6,000° C., being attainable. In the molecule of water we may consider that the electrons of the hydrogen atoms have been drawn toward the oxygen atom which has a greater attraction for the electrons than the hydrogen proton has, and the molecule $H^+-O^{--}+H$ is formed. Already in 1881 Helmholtz showed that if we assume that electrical forces hold two positive atoms of hydrogen bound to a negative atom of oxygen in a molecule of water, the force of attraction between the hydrogen and the oxygen would be 21 billion times as great as the gravitational force would be. We know now that the electrical forces of attraction between atoms do indeed account for the existence not only of water but also of the hundreds of thousands of combinations of elements which form the world and are formed in the earth's crust and in the teeming life on its surface.

Oxidation.—Space permits us to add here only that when we burn fuel in an engine or food in our own bodies by a process called oxidation, the process consists fundamentally in the shifting of the electrons from the carbon atoms in the fuel to, or toward, the oxygen atoms absorbed from the air, with subsequent combination of carbon and oxygen to form carbon dioxide, very much as in the case of water as described above. Energy is thus made available as in an electrical battery. Again, glucose is formed in our bodies from the sugar and starch we eat; the distribution of electrons in every molecule of glucose is such as to make the molecule the equivalent of a tiny electrical cell and there are untold

myriads of these at the disposal of the body. We shall learn presently (Chapter III) that the stored energy of these minute electrical cells represented by glucose is used by our muscles to do their first work. Some of the sugar is then consumed with the aid of the oxygen taken up by our lungs, and carbon dioxide and water are formed and a new supply of energy made available. That a shift of electrons takes place from the carbon atoms of the sugar to the oxygen atoms of the air absorbed by the blood from the lungs, may be demonstrated by the production of a current of electricity from glucose and oxygen under conditions approaching those of the body.* Dr. Murlin's article on *The Body as a Chemical Engine* brings out indeed the fact that the energy available in our bodies seems to be rather the store of energy of electrical cells than that of a fuel engine.

Some of the next great advances in the service of chemistry to medicine are bound to come from applications of the knowledge of the electrical structure of atoms. Particularly inviting is the study, from this point of view, of colloids (jelly-like masses and suspensions), of which our bodies consist, as will be discussed presently. There are electrical charges on suspended bacteria and on other suspended particles and it is already known that such charges have a great deal to do with the behavior of colloids. Our cells are of this kind. The charges have their origin in the electrical structure of atoms.

Synthesis.—It is evident that in the work of discovery by analysis the chemist follows the plan which an

*A detailed discussion of this problem is to be found in the booklet on "Chemistry and Recent Progress in Medicine," the Charles E. Dohme Memorial Lectures of 1924 at Johns Hopkins University, Williams and Wilkins Press, Baltimore, Md.

intelligent architect would pursue in studying any impressive monumental structure: he would study the general plan of the building in detail; he would consider the individual materials out of which the building is constructed—marble, brick, steel or wood; he would study the way these materials are combined; in a word, he would study the structure of the building as minutely as the chemist studies the structure of a molecule. With this knowledge he will be prepared to construct the edifice himself and may even undertake to build something decidedly better than the original. Now, this is exactly what the chemist often does in his field, once he has made his exhaustive examination of any given principle. What he has taken apart by analysis, he is sooner or later led to attempt to put together again. This effort to build up by synthesis is the significant characteristic of the second great method of attack by chemistry on problems presented to it for solution.

Creative Chemistry.—Synthetic or creative chemistry has added almost unlimited power to the possibilities of chemistry to serve man both in medicine and in industry. Space permits the illustration of this power only by its application to a very few problems. In the first place, knowledge of the composition and structure of epinephrine has made it possible to prepare epinephrine artificially from coal tar products; we are no longer dependent on animals as a source of supply. Harington in London has recently prepared synthetic thyroxine identical in every respect with the natural principle. The preparation from animal sources is very expensive on account of the very small amount present in each animal; the synthetic preparation should ultimately make the product much cheaper. It is significant that this success in

preparing artificial thyroxine followed very quickly upon the successful determination of the molecular structure of the principle. Before that, every effort to prepare synthetic thyroxine had failed. Such instances bring home to us the importance of these difficult and patient inquiries into the structure of molecules. The isolation of pure insulin by Abel from the commercial preparations should lead ultimately to the determination of its molecular structure and then to its artificial preparation. Insulin is now so expensive that a cheaper product would be a great blessing to thousands of diabetics.

In these instances, the chemist has tried only to duplicate what nature has offered to man. But his power goes even further, he can often improve on nature. Thus, cocaine was the first drug used to produce local anesthesia by deadening, when injected, the sensitiveness of tissues to pain. But cocaine has always had certain serious drawbacks: it is very expensive, because rare; it is quite poisonous and has often enough caused death, even in a dentist's chair; as is well known, it is habit-forming, and the cocaine habit is one of the most tragic causes of human wreckage. The determination of the exact structure of the molecule of cocaine by analytical methods was a very long and very difficult undertaking. But once it was accomplished, it made possible the artificial preparation of similar compounds, modified until finally the best qualities of cocaine were preserved and most of its drawbacks removed—exactly as the well informed breeder improves his stock by selection. A local anesthetic such as procaine (novocaine), evolved as a result of this planned work, is as effective as cocaine, it is much less dangerous, and it is not at all habit-forming (see p. 465).

Future Progress.—In time other undiscovered active principles essential to life will be isolated; they are clearly most difficult to obtain in a pure condition from natural sources. Analytical processes must lead to their discovery and separation and ultimately to an intimate knowledge of their structure; constructive synthesis will in many cases be called upon to supply man with these essentials of life cheaply and in quantity. Such principles affect character and mind as much as they do bodily physique as is shown by pictures in Dr. Kendall's article (p. 234). The possibilities inherent in supplies of these powerful pure principles must be particularly attractive in this humane and democratic age which is striving for equality in opportunity in the widest sense. With the aid of chemistry, modifications of the hormones no doubt will also be attempted cautiously and directed toward improvement. This may open new paths for betterment both of human life and of our domesticated breeds of animals.

That improvement in drugs other than the natural principles and the discovery of entirely new synthetic drugs have already progressed very far indeed through the analytical and synthetic efforts of chemistry in cooperation with medicine is the clear note of many of the articles in this book. These include the articles dealing with anesthetics, hypnotics, the treatment of pus infections, of syphilis, leprosy and other diseases. The hopes for the future in this respect are outlined most forcefully in the last chapter.

Physical Chemistry.—Analytical and creative chemistry do not exhaust the fundamental methods of attack on problems by chemistry. Isolation of pure principles, determinations of their molecular structure and artificial

preparations are important but they are only part of the story. A clever engineer can take an automobile engine apart and put it together again; but progress in automobile construction depends ultimately on expert studies of the functioning of all of its parts. The efficiency with which the chemical energy supplied to the engine in the form of gasoline is converted into work is a criterion which every engineer must keep in mind. In a similar way, chemistry brings to bear on the study of life and all other problems which it faces, its knowledge of the laws governing the functioning of chemical substances. This important field of chemical effort is the particular interest of what we call *physical chemistry*.

The study of all energy changes connected with chemical processes is the special concern of the physical chemist and it is by virtue of such energy changes that we keep ourselves warm and do our work. Physical chemistry also studies the laws determining the distribution of matter and the behavior of matter as affected by foreign surfaces, somewhat as the automobile expert is concerned with lubrication of moving parts as well as with the distribution say of gas and air in an explosion chamber. Of particular importance to life is the study of the so-called colloidal condition of matter in fine suspension as we see it in jellies, in fresh milk, milk of magnesia, or froth. The human body in its tissues, organs and blood is a tremendously complex colloidal system. Very small changes disturb such suspensions: souring coagulates milk, time or churning separates the cream and finally the butter from milk. The colloidal systems of the body are equally sensitive to forces affecting their functioning and stability. Our kidneys are sensitive to acid and to many poisons even in small

quantities; coagulation of blood in an artery or vein forms a clot that can stop the heart or destroy the functioning of the brain; the rigor of death is the result of widespread colloid changes in the body. The vital importance of colloidal behavior in the functioning of the body is emphasized by many of our contributors. The physical chemist is penetrating ever deeper to a knowledge of the behavior of colloids, but here again there are very great gaps in our knowledge, which must be bridged before medicine can have the aid it needs in the study of many of its problems.

Two or three of the simpler practical applications of physico-chemical methods of attack must suffice for our present purposes. In the first place, our health evidently depends on the efficiency with which the human engine works; this is dependent on many factors but the net result of their effects may be found in a test of what is called the basal metabolism of the body. This measures the ability of the body, when at rest, to utilize food ingredients adequately in the tissues. For practical purposes, the physician studies the basal metabolism of a patient by determining the ratio of oxygen consumed by the patient to the intake of oxygen in the air breathed. An exhaustive examination of a patient for symptoms of disease very commonly now includes a determination of this basal metabolism. There is a certain latitude in the normal found in ordinary good health and allowance is made for age, sex and weight. Serious deviation from the norm is a sign of present or impending trouble—such as too much or too little thyroid secretion. An early warning arrived at in this way very often saves the patient from more serious trouble later.

Again, blood examinations for sugar, alkalinity,

alkali reserve and other factors are of value only as judged from a comprehensive knowledge of blood equilibrium and the functioning of the blood in such conditions as diabetes, gout, acidosis, anemia, etc.

Finally, we have this illustration: Asphyxiation by carbon monoxide has long been a menace to life. Formerly it was found chiefly in the closed coal or charcoal furnace, when the fuel burned with insufficient air to consume the monoxide (CO) and form the far more harmless dioxide (CO_2). Today our automobiles pour out the poisonous monoxide with every throb of their engines. To remain with a running engine in a closed garage is suicidal. Carbon dioxide which we exhale from our lungs as the final product of combustion of the carbon in our food is also asphyxiating but only in a highly concentrated form. The oxygen in the air is life sustaining. The natural course of treatment that first suggested itself in cases of asphyxiation by carbon monoxide was the inhalation of pure oxygen, usually with the aid of a pulmotor. This treatment was not always effective and a better procedure was discovered by Yandell Henderson of Yale University and his associates. They called attention to the fact that respiration in the body is stimulated chemically by carbon dioxide. a waste product of respiration itself—remotely as glowing ashes facilitate the burning of fresh fuel on the hearth. Careful experimentation determined the correct proportion of carbon dioxide to be added to pure oxygen to excite the lungs of the victim of asphyxiation to normal respiration. As a result more lives are being saved from the deadly menace of asphyxiation than could be saved before. In steel mills carbon monoxide may escape from the furnaces in so great a quantity that two or

three scores of men are liable to drop in a very few moments. In preparation for such a contingency an up-to-date steel mill has prepared for immediate use sufficient oxygen containers, carrying just the right proportion of carbon dioxide, to save life on a large scale with the utmost dispatch—for delay means a far greater hazard.

Physical Chemistry in Isolating Pure Principles.—Further illustrations of the importance of the physico-chemical method of attack on medical problems will be found in many of the chapters that follow. Some of the most serious difficulties facing medicine at present will be overcome only with the aid of this method of approach to the problems. Thus, the dangers of anaphylactic shock in the use of antitoxins, involving even death, have been emphasized in our introduction. Nevertheless, the antitoxins are indispensable in saving life in diphtheria, lockjaw and other most dangerous diseases. The grave risk in their use is caused not by the active antitoxins, whose dosage is accurately measured, but by accompanying proteins of the vehicle, usually horse serum. The greatest progress toward the isolation of pure antitoxins is being made through the application of physico-chemical methods of work. The astonishing success of Dr. Abel in obtaining the pure crystallized principle of insulin was in large measure the result of the application of physico-chemical methods to the problem by this brilliant investigator. The whole world of science had already been working for some years in feverish haste to isolate the pure principle from insulin preparations when Dr. Abel started his work. Within a few months he solved the great problem by a plan of attack based in part on physico-chemical principles.

Future Progress.—Does this brilliant campaign not hold forth the hope, nay the promise, that other wisely planned investigations, applying the facilities of every branch of chemistry, will eventually result in the isolation of all the remaining important hormones of the body? Of these perhaps the sex hormones are the most important and interesting (p. 256). Will they be found to contribute to the preservation of health, to the preservation of the courage, working power and speed of early manhood, so that men can continue to work at their best for more years and finally drop in harness? Will they help medicine in the treatment and cure of certain forms of insanity, the most terrible of all diseases, which is taking an ever larger toll of our people, both young and mature?

These are only some of the outstanding unsolved great problems of human happiness as based on human health. To their solution chemistry must contribute its services to medicine along all of its lines of attack: searching analytical methods, comparable with the work of pioneers, scouts, observers of every kind in warfare; its creative powers to improve known curative agencies and recruit new remedies, comparable with such advances in warfare as the invention of the machine gun, the submarine, the aeroplane and the tank; and finally its physico-chemical methods of studying and controlling functions, which are comparable with the skill of engineers in providing at the right place and time for the successful movements of large forces, for making possible such coordination of effort as finally leads to a victorious advance. An army staff will utilize and organize all of these forces and countries spend annually hundreds of millions in these directed efforts which

eventually end in the destruction of life. Will it not pay every country to organize equally effective cooperation between medicine and the fundamental sciences in the nobler effort to save and preserve life with a tithe only of the expense now devoted to preparations to destroy? The answer to this question will be found, it is hoped, in the chapters that follow.

CHAPTER II

Heredity and Development

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THE word heredity is often used in a sense that is figurative and inexact. We speak of inheriting bodily and mental traits as we speak of inheriting an estate. But as a matter of fact children do not receive traits from their parents, for parents continue to possess their traits after the children are born. Traits are not transmitted at all: they are formed anew in each generation. What is passed on is merely two cells, an egg cell from the mother and a sperm cell from the father; and every individual originates from the union of these two.

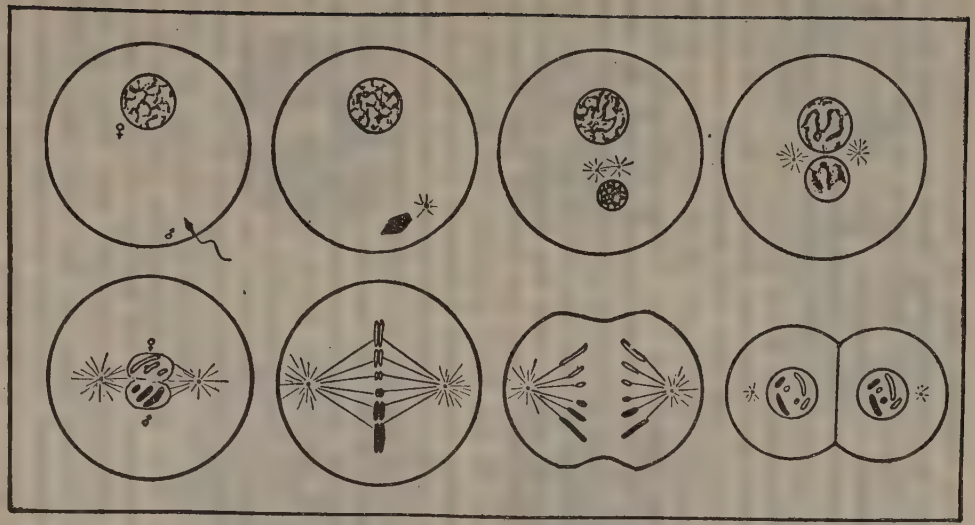
Since on the average children inherit equally from their two parents, the egg and the sperm must contribute equally to the formation of the new individual. But in appearance these two cells differ greatly. The egg is round and consists of cytoplasm within which are embedded a number of bodies, often rod-shaped, called the chromosomes. In the human egg there are twenty-four chromosomes; in other species the number varies from one in the egg of the parasitic worm Ascaris

to several hundred in some protozoa, but in any one species the number is constant. The sperm also contains a constant number of chromosomes, generally the same number as the egg. But it contains little else. Its chromosomes are closely packed together and constitute the head of the sperm; what cytoplasm there is forms a thin envelope about the head and a long tail which propels the sperm up the oviduct. Between the tail and the head is a small body called the middle-piece.

The fact that the egg and the sperm exert equal effects would seem to indicate that their influence depends not on the cytoplasm, which is so much larger in the egg, but on the chromosomes, which are equal in number in the two cells. Modern genetical research has completely substantiated this conclusion. It has been shown that the units of heredity, or genes, are located in the chromosomes; and that within each chromosome they are arranged in a line. The chromosomes in an egg or a sperm cell are qualitatively different from one another. For every chromosome in the egg there is in the sperm a corresponding chromosome composed of similar genes similarly arranged.

In the process of fertilization, the sperm head penetrates the egg, introducing the paternal set of chromosomes. The tail and middle-piece may enter also or they may remain outside. Each chromosome maintains its individuality; hence the fertilized egg cell has two complete sets of chromosomes: in the human species the number is forty-eight.

In the cytoplasm of the fertilized egg there appears, in the neighborhood of the sperm, an aster, which is composed of a central body (centrosome) surrounded by radiating lines. The aster divides into two, and



Courtesy of the McGraw-Hill Book Company.

PLATE I.

DIAGRAM OF FERTILIZATION AND CLEAVAGE OF THE EGG. (*After Sharp.*)

these move to opposite sides of the cell. Lines called spindle fibres appear extending from one aster to the other. The chromosomes (both those of the egg and those introduced by the sperm) range themselves between the asters in a plane perpendicular to the spindle fibres; each chromosome splits longitudinally; and the two halves separate, one going toward each centrosome. The cytoplasm then constricts between the two groups of chromosomes; and two cells result, each containing the same number and kinds of chromosomes as the fertilized egg (see Plate I). These two cells remain in contact with each other. In each of them the aster, chromosomes, and cytoplasm divide again in the same fashion as before, so that four cells result; and by this process repeated many times the myriads of cells that make up the human body are formed (see Plate II).

Every cell of the body contains two complete sets of chromosomes, one derived through the egg from the mother, the other through the sperm from the father. With respect to the chromosomes, therefore, the cells are all duplicates of one another. But they come to differ greatly in their cytoplasms: some develop into muscle cells, others into nerve cells, still others into gland cells, etc. We are therefore confronted with several problems. (1) How does the sperm activate the egg? (2) Why do the fertilized egg and the cells derived from it divide? (3) Why does the organism so formed grow? (4) Why do the cells arrange themselves in the pattern characteristic of the species? (5) Why do they change as development proceeds? (6) Why do they change in different ways so that they come to differ from one another? (7) Why does the individual resemble its parents in so many ways and differ from

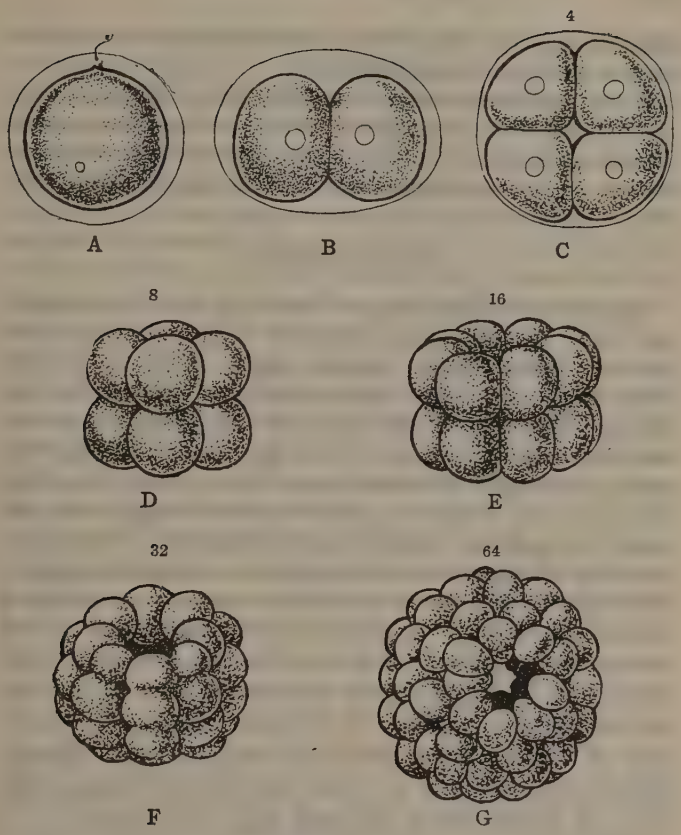


PLATE II.

CLEAVAGE OF THE EGG. A, EGG AND SPERMATOZOA BEFORE FERTILIZATION. B-G, SUCCESSIVE STAGES IN THE DIVISION OF THE FERTILIZED EGG. (*After Selenka.*)

them in others? (8) Why does development come to an end and death finally ensue?

These problems are not independent of one another, and doubtless a complete solution of any one would include solutions of all the others. But we may (if only because of the incompleteness of our knowledge) treat them separately.

DEVELOPMENT

Activation of the Egg.—The egg can be activated not only by the sperm but by many chemical and physical agents (acids, alkalies, salts, electricity, etc.). This shows that the chromosomes of the sperm, although they carry the paternal inheritance, are not necessary for activation. It has been supposed that the sperm introduces the centrosome of the aster; however that may be, asters are also formed in eggs that are made to undergo artificial parthenogenesis. The great diversity of agents that bring about activation suggests that they (the sperm included) merely set going a mechanism already contained in the egg. The cortex of the egg cytoplasm is a factor in the process; for an egg can be activated if part of its cytoplasm is removed but not if it is deprived of its entire cortex. The presence of various substances in the egg has been demonstrated chemically or by their effect on the sperm; but it is not known what rôle they play in activation.

Cell-division.—Various physico-chemical changes can be shown to take place during division. The reactions of the chromosomes to stains alter; changes in the viscosity of the cytoplasm occur, the asters becoming relatively solid and the region between them more

liquid; the surface tension increases at the equator and decreases at the poles. Gurwitsch has reported that some cells give off rays in the ultra-violet part of the spectrum and that these "mitogenic rays" stimulate division in other cells. The changes in viscosity and surface tension may explain at any rate in part the division of the cytoplasm. It has been suggested that the chromosomes are pulled apart by the spindle fibres, since they seem to be attached to the fibres and their separation can sometimes be observed to begin at the points of attachment. It has also been suggested that electric and magnetic forces are involved; and there is evidence that a difference in electric potential exists between the chromosomes and the cytoplasm. But even if these explanations apply to the separation of the chromosomes and of the asters, the splitting of a chromosome or a centrosome into two remains unexplained.

Growth.—The splitting of a chromosome involves a division of each gene; so that the relative amounts of different genic substances remain approximately constant, and when growth occurs the total amount of genic material is increased. This means that substances in the cytoplasm are transformed into genic material. The transformation must be brought about under the influence of the genes themselves; for when (as occasionally happens) a gene is altered, say from A to A′, the cytoplasmic substances continue to be transformed but are now changed into A′ instead of A. Each gene produces a reaction of which it is itself an end-product; that is, the gene is autocatalytic.* In plants there are, in addition to

*A catalyst is an agent causing or accelerating a characteristic chemical change.

the chromosomes, bodies in the cytoplasm which also reproduce by division and persist from one generation to another; these bodies, called plastids, are therefore also autocatalytic. Chromosomes and plastids resemble enzymes (like pepsin, etc.) in their catalytic activity, and bacteriophage in being autocatalytic. It has, in fact, been suggested that genes are enzymes and that bacteriophage is composed of free genes; but these hypotheses cannot at present be tested, since we cannot analyze the substances chemically.

The genes are a factor in development, for on the rare occasions when a gene in a cell is altered or lost, the characters of the cell are modified. The growth of the cytoplasm may therefore be looked upon as a mere by-product of the activity of the genes. We may paraphrase Samuel Butler's statement that the hen is only an egg's way of making more eggs, and say that the organism is only the genes' way of making more genes.

Arrangement of Cells.—How cells are arranged is determined partly by their positions when formed, partly by their subsequent movements. The original arrangement of two cells formed by division depends on the position of the spindle in the cell from which they are derived; and this in turn depends on various factors. The direction of the spindle is often at right angles to that of the spindle in the previous division. But this is not invariably true, for in some cells division always takes place in the same direction. The orientation of the spindle can be altered by pressure; if a cell is compressed in one direction (and therefore elongated in another) the division furrow is formed at right angles to the long axis. There must be many special conditions

that affect the position of the spindle, for the cleavage patterns of different species are very diverse.

Ultimately the diversity must be caused by differences in genes; and in one case this has been demonstrated. In certain snails there are two types of individuals: some shells coil in a right-handed, others in a left-handed spiral. The cleavage patterns of these two types are asymmetrical and (like the shells) mirror images of each other. They are determined before the egg is fertilized and the difference between them is due to a difference in a single pair of genes. How the genes produce these effects is not known, but a possible interpretation will be considered later.

Cleavage patterns can be imitated by arrangements of soap bubbles or drops of liquids, and some of their characteristics can be explained by the laws of surface tension. Another factor in the arrangement of cells is the chemical nature of the environment. Lack of calcium in sea-water in which marine eggs are developing causes the cells to separate instead of remaining attached. In fishes developing in sea-water to which magnesium sulphate has been added, the two eyes coalesce into a single median eye. Tactile stimuli may also play a part: nerve cells *in vitro* grow not into free space but along solid objects, like organisms that are positively stereotropic. Doubtless cells react to various environmental stimuli —tactile, electrical, chemical, etc.—in the same way as do organisms; the similarity in behavior is sometimes (as in the case of the white blood cells and amoeba) very striking; and further research may be expected to analyze cellular responses into tropisms and motor reflexes. Differential rates of growth must also be taken into account. If two contiguous layers of cells

grow at different rates, bending will take place toward the layer that grows less quickly; and in general the shape of an organism depends on the rates of growth in different directions. Cells may also be carried passively by the blood or lymph; cancer cells, for example, may be transported in this way.

Genetic Constancy in Development.—The cells of the body differ not only in position but also in size, shape, and chemical constitution. These characters are cytoplasmic; and since the cytoplasm is built up under the influence of the genes, it might be supposed that the diversity of cells in an individual is due to differences in genes. There might be a sorting out of genes, so that the cells would not all receive the same combination; or the genes might change, and change in different ways in different cells. These hypotheses, even if they were true, would still leave unexplained the question why the sorting or modification follows the pattern characteristic of the species. But with this problem we need not concern ourselves, since neither hypothesis is in accordance with the facts. The first is contradicted by the accurate lengthwise splitting of each chromosome in division, for this would seem to involve a division of each gene. The second is contradicted at least in part by the facts of heredity; for at any rate in the germ cells and the cells from which they have been derived, the genes are unaltered except for rare changes called mutations. Development cannot, however, be ascribed to mutations since it proceeds even if no mutations occur.

Moreover, embryological experiments indicate that in general neither a sorting out nor a modification of genes takes place; for in many cases all the cells of the embryo, at least up to certain stages, have the same potentialities

as the fertilized egg cell. In the sea-urchin, for example, if the cells of the embryo are separated in the two- or four-cell stage, each develops into a complete larva.

The development of an egg into more than one individual is the rule in some animals, where however it is due not to the separation of cells but to a budding of the embryo. In the armadillo every embryo forms four buds and produces quadruplets which are of the same sex and resemble one another very closely in other ways; this again demonstrates that the four parts receive the same complement of genes. A similar process seems to occur occasionally in man: twins that are of the same sex and otherwise so similar that they are difficult to tell apart (identical twins) are in all probability derived from a single fertilized egg. Not all twins, however, are identical: some (and this is true also of multiple births in most mammals) originate from different eggs that happen to have been liberated simultaneously; they may be of the same or of different sexes and the resemblances between them are no greater than those between ordinary brother and sister.

In some organisms the potentialities of cells are not limited even in later stages. A planarian worm cut into several pieces produces a complete individual from each piece. There are cases of regeneration in which cells of one tissue are transformed into different tissue; and this indicates that their potentialities have not been limited by their cytoplasmic differentiation.

The unlimited possibilities of many cells are also shown by the fact that, when transplanted from one part of an embryo to another, they develop not like the cells from whose midst they were taken but like those amongst which they have been set down. Cells that

would, in the ordinary course of events, have become skin, may thus be caused to develop into nerve or eye lens or other tissue; and similarly prospective nerve or lens cells may be transformed into skin.

Every cell, then, seems to receive all the genes and to receive them unchanged. What a cell develops into depends on its location in the embryo; and apparently different parts of the embryo exert different influences. Why, then, are these influences different? Obviously because the parts are different. But why are the parts different? That is exactly what we started out to discover, and we seem to have gone the long way round only to come back to our starting point.

We have, however, learned one thing on the way; namely, that the fate of a cell depends on its environment. It may seem at first sight that the environment of the cells of the embryo does not alter, and certainly does not alter quickly enough to account for development. It may seem curious too to speak of environmental differences within a region as small as that occupied by the early embryo. Nevertheless observations and experiments show that the environment of the cells does change and that environmental differences do exist not only for different cells but even for different parts of the egg before fertilization.

Differentiation Dependent on Environment.—While the unfertilized egg is still in the body of the mother, it is attached at only one point; hence the parts of the surface are not in equal contact with the maternal tissues. The non-uniformity of environment has been shown to affect the distribution of cytoplasmic substances: in the sea-urchin egg, for example, these become arranged in parallel layers perpendicular to that diameter of the egg

which passes through the point of attachment. The point of attachment itself becomes the animal pole (that is, the point at which the polar bodies are given off); and since in normal development the animal pole becomes a definite part of the embryo, some of the differences between the parts of the body may be traced back to environmental differences to which the unfertilized egg is subjected.

The differentiation into dorsal and ventral regions or (what comes to the same thing) into right and left sides is, in some animals (insects, squid), also determined in the unfertilized egg while it is still in the body of the mother; but in other species this is not accomplished until later. In the frog it has been shown to be connected with the entry of the sperm, for the plane dividing right from left, which passes through the axis of radial symmetry, passes also through the point at which the sperm enters.

Bilateral symmetry may be determined in still other ways. In natural and artificial parthenogenesis the egg develops without the entrance of a sperm. When parthenogenesis is induced in a radially symmetrical egg like that of the frog, bilateral symmetry is determined presumably by some local influence; but it is not known what this influence is.

Changes in the environment of cells are also brought about by the division of the egg. For the resulting cells are in contact with one another instead of being free like the egg cell. This explains why each cell, despite the fact that it contains the same genes as the egg, develops into only a part of the body. For if the contact is removed by an artificial separation of the cells, each cell will, in some species, develop into a complete embryo.

How the contact acts is not known. It may produce or maintain a non-uniform distribution of substances. At any rate if the frog's egg after the first division is turned upside down so that in each cell the contents are redistributed, each cell develops into a whole individual.

As cell division proceeds, there is a greater diversity of relations of the cells to one another and to the external environment. Only the outermost layer of cells is in direct touch with the medium surrounding the embryo. Some equalization is brought about by the nerves and blood vessels: for these, in the embryo as in the adult, are paths by which external agents may affect parts that would otherwise remain beyond their influence. But nerves and blood do not supply all parts uniformly; hence cells are subjected to different stimuli and the supply of oxygen and other substances and the removal of waste materials are not the same for all of them.

Once local differences have been established, the parts of the embryo may affect one another chemically as in the familiar instance of internal secretions. The importance of these in development is seen in such facts as the abnormalities induced by lack of thyroid (cretinism) or oversecretion of pituitary (gigantism). Such influences are not limited to glands. In many amphibia the lens of the eye is formed under the influence of the optic cup, for if the latter is artificially removed or caused to develop too far below the surface, the skin cells that would have formed the lens fail to do so. Moreover, if the optic cup is transplanted into another region of the embryo, the skin cells of that region are transformed into a lens. The nature of the influences in these cases is not

known, but is presumably chemical. Thus complexity may beget more complexity.

The influence of the parts of the body on one another persists even into the adult stage, for some cells removed from the body and grown *in vitro* undergo changes that have been termed dedifferentiation. And the regeneration of lost parts shows that before they were severed the parts exercised an inhibitory effect on each other's growth.

In the embryo, as after birth, the development of an organ must be affected by its functioning, and also by changes in the external environment. Even the mammalian embryo, which develops at a uniform temperature in the body of the mother, must be influenced by the modifications which the maternal metabolism undergoes during pregnancy.

Differentiation Apparently Independent of Environment.—The cases so far considered are, however, only a selected group. There are others in which development seems to take its course regardless of environmental changes. In many mollusks, if the cells of the early embryo are separated from one another, each forms not a complete embryo but the same part that it would have formed if it had remained in contact with the others. Here it seems as if differentiation depended on some internal pattern of the egg which is already determined when cleavage takes place. An internal pattern is in fact visible, since there is a definite arrangement of visibly different substances; but this cannot be the pattern involved, for the rearrangement of these substances by means of centrifuging does not modify development. On the other hand, the centrifuge would be expected to have no effect on a pattern if the latter depended

on the arrangement not of visible particles but of the molecules themselves, as does the structure of a crystal. The comparison between organisms and crystals is also suggested by the two types of spiral cleavage in snails: these are mirror images of each other and might be due to cytoplasmic substances whose molecules were also mirror images. If such substances exist they must be in the cytoplasm, because an enucleated fragment from any part of the egg cleaves, if fertilized, in the same way as the egg from which it was taken. But the substances must be produced by the genes or under their influence, because the type of pattern is inherited through both egg and sperm; and this suggests that the differentiating genes, or parts of them, may also be mirror images of each other. These facts argue that cytoplasmic patterns are formed anew in each generation and do not persist as such from one generation to another. Whatever may be the explanation of spiral cleavage in snails, not all developmental patterns are due to molecular arrangement: in the frog's egg, as we have seen, the pattern can be altered by merely inverting the egg. A molecular pattern should be distinguishable from one whose elements are larger because the former should remain unaffected when the cytoplasm is mixed by centrifuging or by the high-frequency sound waves studied by Langevin and by Wood and Loomis.

A cytoplasmic pattern dependent on the molecular arrangement of substances produced by the genes might be formed even in a uniform environment. But it does not follow that this interpretation is correct even where development is uninfluenced by experimental procedure. For these experiments prove only that the developmental pattern is not modified by the particular environ-

mental agents used at the particular time they were applied. It is entirely possible that the pattern might have been altered earlier or by different agents, and that it was laid down in the first place under the influence of environmental differences.

The difference between the two types of development that we have been considering may therefore turn out to depend merely on the time at which the fate of the cells becomes definitely determined. Even where the potentialities of cells are unlimited for a long time, they do not necessarily remain so indefinitely. It is true that in planarians no limitation seems to occur; but in other animals a time comes when the cells seem to have assumed their final forms for good, or at any rate for better or worse. In the amphibia a piece of tissue transplanted after a certain stage develops as it would have in its original position. Cancer cells maintain their form even if transported to tissues different from those in which they originated. The stages at which cells assume a definitive form may differ even in closely related species: the lens of the eye, which in some amphibia (as we have seen) begins to develop only in the presence of the optic cup, in other amphibia begins to form regardless of whether the optic cup is present or not. This does not necessarily mean that in the latter species the cells from which the lens is produced are not susceptible to environmental stimuli; it may mean merely that the stimuli involved have already acted and produced their effects.

On the other hand, development in all cases must be, to some extent, independent of environment: since even if the environment remains unaltered, one chemical reaction within an egg or embryo may lead to another.

The limitation of the potentialities of cells is in itself an interesting and important problem. Theoretically it might occur either because the cells involved had lost their power of response, or because suitable stimuli were no longer presented to them; or for a combination of these reasons. The experiments of Carrel and his associates, which have shown that inert cells from old animals may be stimulated to active division by embryonic juice, suggest that the relative constancy of cells in the adult may be due to a lack of stimuli. But since the stimuli themselves are produced by cells, the problem is merely shifted: instead of inquiring why some cells have altered so as to become unresponsive, we must ask why other cells have altered so that they no longer exert the same stimuli.

Ageing.—We know a good deal about the changes that occur when an organism ages, and something about the processes by which these changes are brought about. Some of the wasting of old age is due to phagocytosis, a process that causes similar modifications (like the absorption of the tail in the tadpole) earlier in development. Whether old age and death are the inevitable result of developmental processes, we do not know. Single-celled organisms, which reproduce by division, seem to be potentially immortal; and the same is true of the germ-cells of many-celled animals. But it is not known whether the many-celled organism could maintain the balance of vital processes indefinitely.

HEREDITY

The rule that all the cells of an individual contain identical sets of genes is not without exceptions. Chromosomes sometimes lag during division and are

lost; or two chromosomes that ordinarily separate may go to the same cell; or a chromosome may fragment and part of it may be lost or attached to another chromosome. In this way cells may come to differ in their genes and therefore in the characters they display. But such occurrences are accidental and relatively rare, so that normal development cannot be attributed to them.

In some cases there is a regular elimination of chromatin in somatic cells. In the worm Ascaris, all cells except those that give rise to germ-cells extrude part of each chromosome into the cytoplasm, whereas the germ-cells retain each chromosome entire. This phenomenon, although it may account for the difference between soma and germ, does not explain why the somatic tissues differ from one another. Complete extrusion of chromosomes occurs in the red blood cells of mammals, which lose their entire nucleus. Such procedures, however, are very uncommon.

Segregation.—But there is one kind of sorting out that occurs in all animals and plants. It takes place in the germ cells before fertilization. In the last division of a germ cell or the one before the last, each chromosome does not separate into two: instead the two chromosomes of a pair lie side by side on the spindle and separate from each other, one going to each cell. As a result of this segregation the ripe egg or sperm contains half as many chromosomes as the other cells of the body—only one of each pair instead of two. The double number is restored by fertilization.

The segregation division is the same for the cells that give rise to eggs and those that give rise to sperm so far as the chromosomes are concerned. But there are differences in the behavior of the cytoplasm. In the forma-

tion of the sperm the cytoplasm divides equally, and both cells formed are functional. In the formation of the egg, however, the division of the cytoplasm is unequal so that one daughter cell receives almost all of it and the other almost none. The large cell becomes the egg; the small cell (known as the polar body) usually degenerates and takes no further part in development.

In the formation of both egg and sperm the segregation division is either followed or preceded by another in which the cytoplasm divides in the same way—that is, equally in the sperm and unequally in the egg. In this division, however, the chromosomes do not segregate but divide as in ordinary divisions, so that the distribution of genes is not affected.

Segregation of the chromosomes is of little importance in development, since it occurs only in the germ-cells and even there takes place only in one of the last two divisions—by which time, in many species, the egg has already been extruded from the body. But it is very important in heredity, because it involves a segregation of genes. Each ripe germ cell (unlike the other cells of the body) contains only one gene of each pair; and the sorting out of the genes determines what each egg and sperm is to carry over to the next generation. The segregation of the genes can be followed by observing the inheritance of characters; and it was in fact discovered by Mendel before the segregation of chromosomes was known.

If an individual is pure for all its genes (that is, if the two genes of every pair are alike), its genetic constitution may be represented as follows:

$$\frac{a\ b\ c\ d\ \ldots}{a\ b\ c\ d\ \ldots}\quad\frac{l\ m\ n\ \ldots}{l\ m\ n\ \ldots}\quad\frac{q\ r\ s\ t\ \ldots}{q\ r\ s\ t\ \ldots}$$

where the genes of corresponding paternal and maternal chromosomes are separated by a line. For simplicity's sake only three chromosomes are represented and in each chromosome only a few genes.

Every germ cell of such an individual will receive one chromosome of each pair: and since the two chromosomes of every pair are identical, it makes no difference which one any germ cell receives. All the germ cells will therefore be alike, their genetic constitution being *abcd . . . lmn . . . qrst . . .*

If, however, the individual is not pure for all the genes, the ripe germ cells will be of more than one kind. Thus if the genes of one pair differ from each other, the genetic constitution may be written as

$$\frac{a\ b\ c\ d\ldots}{a\ B\ c\ d\ldots}\quad\frac{l\ m\ n\ldots}{l\ m\ n\ldots}\quad\frac{q\ r\ s\ t\ldots}{q\ r\ s\ t\ldots}$$

There will now be two kinds of germ cells: some will receive the genes *abcd . . . lmn . . . qrst . . .*, others will receive *aBcd . . . lmn . . . qrst . . .*; and these two classes will be equal in number.

When an individual is pure for all its genes, segregation cannot be detected by breeding experiments, since the germ cells that receive the genes of any one chromosome do not differ from those that receive the genes of the other chromosome of the pair. But when an individual is hybrid, the different kinds of germ cells can be detected by proper crosses, because they will give rise to different kinds of individuals. An illustration is furnished by crosses involving white-flowered and red-flowered races of a plant called the four o'clock (*Mirabilis jalapa*). These differ from each other in a single pair of genes: and (if we omit all the genes which are

identical in the two races) we may designate the white as $\frac{b}{b}$ and the red as $\frac{B}{B}$. When they are crossed, the hybrid receives a *b* gene from one parent and a *B* from the other; its genetic constitution therefore is $\frac{b}{B}$. The flowers of the hybrid are pink in color, and this suggests that there has been a blending of the genes involved. But it can be demonstrated that the genes have not blended and that the hybrid produces two types of germ cells, some carrying *b* and others *B*. For when the hybrid is crossed to a white-flowered plant (that is, when the germ cells of the hybrid are combined with germ cells carrying the *b* gene) the offspring are of two types, white $\left(\frac{b}{b}\right)$ and pink $\left(\frac{b}{B}\right)$. Since all these plants have received *b* from their white parent, the difference between them must be due to their having received different genes from the pink parent: some must have received *b* and others *B*—that is, these two genes have segregated in the hybrid. The fact that half the plants are pure white and if self-fertilized produce only white progeny proves that the gene for white has not been altered by its sojourn in the hybrid.

Similarly, if the pink hybrid is crossed to a red-flowered plant, the offspring are of two equal classes—pink and red. This again proves that segregation has occurred; and since the red plants produce only red progeny if self-fertilized, the gene *B* has not been affected by the gene *b* in the hybrid.

Another example is the inheritance of brown and blue eyes in man. If a person pure for blue marries a person

pure for brown, the children are hybrid for eye color: in this case the hybrid is not intermediate but has brown eyes somewhat like those of one parent. The gene for brown is therefore said to be dominant, that for blue recessive. That the genes do not alter each other is demonstrated by the fact that if the hybrid marries a person pure for blue, on the average half the children will be brown-eyed and half will be blue-eyed. Since all these children have inherited only genes for blue from their blue-eyed parent, the difference in their eye colors must be due to differences in the genes they have received from the hybrid parent: some must have received the gene for brown, others the gene for blue—that is, these genes must have segregated in the hybrid.

Independent Segregation.—Now let us suppose that an individual is hybrid for two pairs of genes in different chromosomes. The genetic constitution may be written as follows:

$$\frac{a\ b\ c\ d\ldots\ \ l\ m\ n\ldots}{a\ B\ c\ d\ldots\ \ L\ m\ n\ldots}\ \ \frac{q\ r\ s\ t\ldots}{q\ r\ s\ t\ldots}$$

When the germ cells are formed, there will again be two types, some receiving the *b*-carrying chromosome and others the *B*-carrying chromosome. But each of these types will be subdivided, since every germ cell that receives *b* will also receive either *l* or *L;* and similarly for every germ cell that receives *B*. There will therefore be four types of germ cells: their genetic constitutions (if we omit the genes that are identical in all of them) may be written as *bl, bL, Bl* and *BL*.

Moreover, these four classes will appear in equal numbers, because a germ cell that receives the gene *b* is just as likely to receive *l* as *L*, and the same is true for a cell that receives *B*. This is due to the fact that the

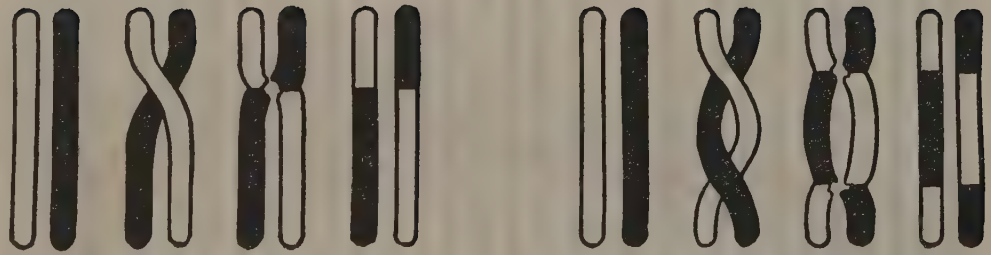
chromosomes of any pair act independently of those of other pairs. When they range themselves on the spindle, it may happen that the chromosome carrying *b* and that carrying *l* are on one side, and the chromosome carrying *B* and that carrying *L* on the other; or it may happen that *b* and *L* are on one side, and *B* and *l* on the other. The first arrangement will result in the germ cells *bl* and *BL;* the second in the germ cells *bL* and *Bl*. Since the two arrangements are equally probable, the four kinds of germ cells will occur in equal numbers.

This is called independent segregation and may be illustrated by a cross involving eye-color and at the same time another character. We may select brachydactyly, an inherited shortness of the fingers due to the absence in each of one of the finger bones. Brachydactyly is dominant over the normal condition. A person pure for brown eyes and for brachydactyly differs from a blue-eyed normal-fingered person in two pairs of genes (which we will assume to be in different chromosomes). If we omit the genes in which they are identical, the genetic constitution of the former individual may be written as $\frac{B}{B}\frac{L}{L}$ and that of the latter as $\frac{b}{b}\frac{l}{l}$. The hybrid therefore will have the constitution $\frac{b}{B}\frac{l}{L}$, and in appearance will be brown-eyed and brachydactylous, since the genes for both these characters are dominant. The germ cells of the hybrid will be of four kinds, *bl*, *bL*, *Bl*, and *BL;* and these will appear in equal numbers. If such a person marries an individual pure for blue eyes and normal fingers, the offspring will tend to be of four equally possible kinds, corresponding to the

four kinds of germ cells of the hybrid parent: blue-eyed normal-fingered, blue-eyed brachydactylous, brown-eyed normal-fingered, brown-eyed brachydactylous.

The principle of independent segregation applies to any number of pairs of genes provided these are in separate pairs of chromosomes. The difference between the skin color of negroes and of whites probably depends on many gene differences; if we assume that three are involved, the negro's genetic constitution may be represented as $\frac{B}{B}\frac{L}{L}\frac{T}{T}$ and that of the white man as $\frac{b}{b}\frac{l}{l}\frac{t}{t}$*. The genetic constitution of the mulatto is therefore $\frac{B}{b}\frac{L}{l}\frac{T}{t}$ and the germ cells will be of eight kinds. For they will be divided into two classes according to whether they receive *B* or *b*; each of these will be subdivided according to whether it receives *L* or *l;* and each of these will again be subdivided into those that receive *T* and those that receive *t*. The eight kinds (*BLT, BLt, BlT, bLT, Blt, bLt, blT, blt*) will occur in equal numbers. Mulattoes marrying among themselves will produce some individuals as dark as pure negroes (if two *BLT* germ cells unite) and some as light as pure whites (if two *blt* germ cells unite) ; but most of the offspring will be intermediate in color, since they will contain genes from both races. Some of them, moreover, will breed true to an intermediate color because the two genes of each pair will be alike (for example, $\frac{B}{B}\frac{l}{l}\frac{t}{t}$). Such cases of "blending" inheritance have been cited as exceptions to segregation; but wherever a critical analysis

*Although some of the letters here used are the same as in previous examples, the genes involved are of course different.



Courtesy of the McGraw-Hill Book Company.

PLATE III.

DIAGRAM ILLUSTRATING SINGLE AND DOUBLE CROSSING OVER. (*After Sharp.*)

has been made it has proved that although the characters appear to blend the genes themselves are not changed but are merely reshuffled.

The examples considered show that for every pair of chromosomes differing in at least one pair of genes, the number of kinds of germ cells is multiplied by two. For n pairs of chromosomes there are 2^n classes of germ cells. In man there are 24 pairs of chromosomes; and if every chromosome differs from its mate, the number of kinds of germ cells that an individual may produce is 2^{24} or more than sixteen million. The great diversity of the human species suggests that individuals are usually hybrid for genes in many pairs of chromosomes.

Linkage and Crossing Over.—But the chances that two germ cells will be identical are even less than these figures would indicate. For the two chromosomes of a pair do not always separate intact. When they lie side by side they twist about each other; and either because of the tension due to the twisting or for some other reason, they sometimes break transversely at corresponding points and interchange corresponding pieces (see Plate III). This process, called crossing over, increases the number of possible combinations of genes many fold and hence decreases greatly the chances that an individual will produce two identically similar germ cells.

The study of crossing over makes it possible to discover the arrangement of the genes in a chromosome, and this turns out to be linear. It is obvious that if three genes in a chromosome lie in a line in the order *A B C*, then (if one break occurs at a time) a break between *A* and *B* will also separate *A* and *C*; and similarly a break between *B* and *C* will separate *A* and *C*. That is, when the middle gene separates from either end gene,

the other end gene remains with the middle gene. But when the two end genes *A* and *C* separate from each other, the middle gene *B* may go sometimes with *A*, sometimes with *C*. This would not be true if the arrangement were not linear: for example, if the three genes were located at the apices of a triangle, any one might separate from the others—that is, there would be no middle gene. By observing the behavior of three genes simultaneously we can discover which of them lies between the two others; and by combining many such observations we can discover the order of all the known genes in a chromosome.

It is, moreover, possible to obtain some notion of the relative distances between genes. For (other things being equal), the longer a distance, the more likely is crossing over to occur within it.

Thus the chromosomes have been mapped. The correctness of the results is indicated by the fact that the lengths of the chromosomes as measured by the number of genes they contain or by the frequency of crossing over within them are proportional to their actual lengths as observed through the microscope. The linear theory is moreover confirmed by the fact that although the frequency of crossing over in a chromosome may be modified by various conditions (genes, the age of the individual, temperature, X-rays), the order in which the genes are arranged remains unaltered. It is true that in rare instances changes in order occur: for example, a group of genes may behave as if it had been transferred to another chromosome. In such cases the group always consists of genes that were consecutive on the map, so that this consecutiveness corresponds to consecutiveness in the actual chromosome; and sometimes an extra piece

of chromosome can be observed microscopically at the point to which the genetic data indicate that the genes have been transferred.

Crossing over may occur at more than one point of a chromosome simultaneously, but the occurrence of recombination at one point tends to prevent recombination at neighboring points. A study of this interference and of the influence on crossing over of genetic and environmental conditions in general has thrown light on the mechanics of the process. But we still do not know precisely what happens when chromosomes break and recombine; nor do we know what causes a cell to undergo a reduction division instead of an ordinary one.

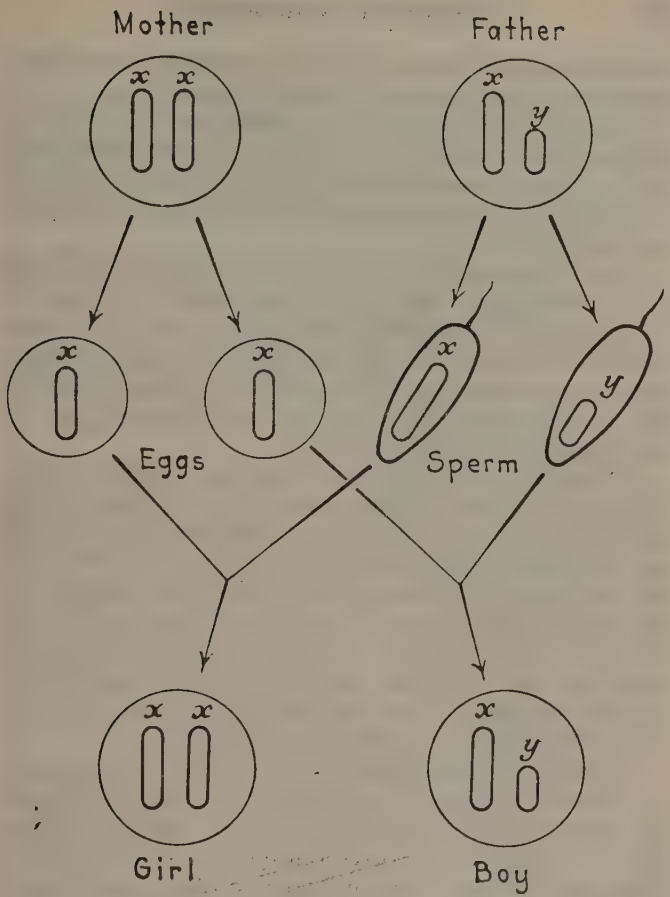
The Inheritance of Sex.—What has been said applies to twenty-three pairs of chromosomes out of the twenty-four in man. But the case of the twenty-fourth pair, which is concerned with the determination of sex, is somewhat different. In the female, the two sex chromosomes (called the X-chromosomes) are alike. In the male, however, the two sex chromosomes differ from each other: one is precisely like the X-chromosomes in the female, the other (called the Y-chromosome) is much smaller. Hence with respect to this pair the constitution of the female is XX, that of the male XY.

When the eggs are formed in the female, the two X's segregate like the other chromosomes and each egg receives one X. When the sperm cells are formed in the male, the X segregates from the Y, so that half the sperm receive an X, the other half a Y. All the eggs are therefore alike, but the sperm are of two kinds. In fertilization two types of combinations are possible. When an egg unites with an X-bearing sperm, the re-

sult is an XX individual, and this is a female; when an egg unites with a Y-bearing sperm, the result is an XY individual, and this is a male. Since the X-bearing sperm and the Y-bearing sperm are produced in equal numbers, the two combinations are equally frequent and the number of females is equal to the number of males (see Plate IV).

In most species so far studied, the Y-chromosome contains no genes that function in development, or very few as compared with the X. This alters somewhat the scheme of heredity. For while in the female each gene in the X has a corresponding gene in the other X, in the male each gene in the X is unpaired, since the Y is virtually empty. Hence the male contains fewer genes than the female and he does not transmit his genes equally to all his sperm; half the sperm receive the empty Y-chromosome and contain only twenty-three chromosome-fulls of genes, whereas the X-bearing sperm receive twenty-four. Consequently although the mother transmits her genes equally to sons and daughters, the father transmits fewer genes to his sons. This fact necessitates some revision of our statement that the influence of the egg and of the sperm are equal in heredity. For while daughters inherit equally from both parents, sons inherit somewhat less from the father than from the mother.

Genes in the X-chromosome are said to be sex-linked. Most of them affect characters other than sex, for example, color-blindness. A color-blind man differs from one with normal color vision in a gene in the X-chromosome. Since the gene for color-blindness is recessive, a woman cannot be color-blind unless she has two such genes (one in each X); she must therefore inherit the



Courtesy of Henry Holt and Company.

PLATE IV.

DIAGRAM TO ILLUSTRATE SEX DETERMINATION IN MAN. (*After Altenburg.*)

genes from both parents, and this explains why color-blindness is less frequent in women than in men.

In view of the facts of sex-linked heredity, the importance often attached to descent in the direct male line is, from a genetic point of view, unwarranted; for a father's X-chromosome and all the genes it contains are lost to his sons.

The Y-chromosome is in some species as large as the X, or even larger; but in the great majority of cases so far studied it is relatively deficient in genes. In some species it is entirely missing and the sperm cells that produce males contain one chromosome less than those that produce females. According to one account the Y-chromosome is absent in man.

The scheme outlined above has been found to apply to mammals, some fishes, most insects, to sea-urchins, and some worms. But in birds, some fishes, and in moths and butterflies, it is the male that contains the two similar sex chromosomes and the female that contains the two which differ; hence there are two kinds of eggs but only one kind of sperm, and the scheme of sex-determination and sex-linked heredity is the reverse of that outlined above. In a few species sex has been shown to be determined by environmental differences.

Exceptional Distributions of Genes.—Occasionally the behavior of genes as indicated by the distribution of traits in heredity is not in accord with Mendelian rules; for example, corresponding genes may fail to segregate. In such cases microscopic observation shows that the chromosomes have behaved in the same exceptional fashion. The identity of distribution in the exceptional as well as in the usual cases proves that the genes are carried by the chromosomes.

THE RELATION OF GENES TO CHARACTERS

Number and Size of Genes.—It has been estimated that in Drosophila a somatic cell in a female contains a minimum of 4000 genes, and in a male (the Y-chromosome being practically empty) 3500. The average size of a gene, obtained by dividing the total volume of the chromosomes by the number of genes, is about 0.06 micron*, a figure that must be considered a maximum both because the estimated number of genes is a minimum and because it is possible that the chromosomes contain material other than genes.

There may seem to be an incongruity in the idea that the characters of an organism depend on such minute bodies as genes. But while each gene is itself small, the aggregate of genes forms a considerable part of each cell. And since between cell-divisions each gene must grow, the proportion of genic material continues approximately constant for the entire organism in successive stages. If we think of the body as being studded with genes, as is literally the case, we shall realize that the amount of genic material is not negligible.

Effects Produced by Genes.—A gene may exert an influence on any part of the body, and at any stage from the egg to the adult. Its effect may be large or small: morphological, physiological, or psychological; normal or pathological. Among human traits that are of interest in medicine the following have been shown to be inherited in a fairly simple Mendelian fashion: albinism; brachydactyly, and a somewhat similar condition known as brachyphalangy; the chemical differences in the blood that determine what bloods may be mixed

*A micron is a millionth of a meter.

in transfusion; sickle-cell anemia; hemophilia (a failure of the blood to clot); color-blindness; night-blindness (total inability to see in dim light); some types of feeble-mindedness; and the insanity known as Huntington's chorea. The inheritance of hemophilia, night-blindness, and color-blindness is sex-linked. Very few other traits in man have as yet been genetically analyzed; but in some other species analysis has proceeded much farther, and in the fruit-fly Drosophila, several hundred genes have been studied and mapped.

The influence of a gene is not limited to one character; in fact a gene seems, in general, to influence several characters. For example, in Drosophila there is a gene which affects the viability of the fly, the shape of the wings, and the fertility of the female. In man, albinism involves a lack of pigment in the skin, hair, and eyes, and a consequent sensitiveness to light; and individuals affected sometimes dye their hair and wear tinted glasses; all these traits, then, may be attributed to a difference in a single pair of genes.

Interaction of Genes with One Another and with the Environment.—On the other hand, a character may be influenced by more than one pair of genes; or, to put it another way, the effect of one gene may be altered or even entirely inhibited by others. Skin color in man is, as we have seen, dependent on at least three pairs of genes. In Drosophila the eye-color is known to depend on over forty pairs, the wings on over ninety. Probably in all these cases many more genes are involved.

Traits are determined not only by the kinds of genes, but also by their relative numbers. The female may contain no genes that are not also represented in the male, for her two X's may be identical with each other and

with his X-chromosome; but the relative numbers are different in the two sexes, because the female has two sex-linked genes of each kind whereas the male has only one. The differences between male and female are, therefore, due to differences in the proportions of sex-linked to non-sex-linked genes. Changes in the number of chromosomes of other pairs, brought about by accidental abnormalities of distribution, also result in modification of the characters of the organism.

Moreover, the effect of a gene may be altered by the environment. There is a race of Drosophila that often have twelve legs instead of the usual six. This race differs from the ordinary flies in a single gene. But the difference in genes produces an effect only if the flies are bred in the cold, for at room temperature the race that is potentially twelve-legged develops only six legs. The coalescence of the eyes of fish embryos in the presence of magnesium sulphate has already been mentioned. In man the color of the skin can be made darker by exposure to sunlight. And many other instances can be given. These cases are only what is to be expected from the facts of development: we have seen that the character of a cell can be affected by its environment; and the organism is merely a group of cells. In addition to these morphological cases, all the physiological responses of the organism to the environment may really be considered as so many instances of changes in the effects produced by genes.

Although in similar environments differences in traits must be ascribed to differences in genes, similarity of traits does not necessarily imply similarity of genes. For if two genes alter so that the effects counterbalance each other, the characters will be unchanged but the genetic

constitution will differ from the original. It follows from this that even in a uniform environment somatic resemblances do not necessarily prove identity of hereditary makeup.

Intermediate Stages between Genes and Characters.— These facts show that there is no one-to-one correspondence between characters and genes. The delimitations between characters are, after all, arbitrary: we may consider the entire arm a single character, or only the hand, or a finger, or a finger-bone. The genes, on the other hand, are actually separate units in the chromosomes. It would be a remarkable coincidence if these units corresponded to the arbitrary distinctions between characters. *A fortiori,* it follows that the genes are not identical with the characters. It is true that their identity has been assumed by some geneticists, and this view has even been imputed to Mendel; but Mendel's paper shows that he was aware of the distinction, since he described the multiple effects of single genes and realized that a character might depend on more than one pair of genes.

There is, moreover, no correspondence between the arrangement of the genes in the chromosomes and that of the characters they affect. In Drosophila, where more genes have been studied than in any other organism, every chromosome contains genes affecting all the parts of the body; and genes located close together may affect very different parts, or may affect the same part very differently. And the position of a gene may alter without in general altering its effect.

The characters, then, are not the genes but are produced by the interaction of the genes with one another and with the environment. A complete solution of the

problem of development would include a statement in physico-chemical terms of all the reactions between the activity of the gene and the final production of the character. Such an analysis is at present beyond our power, but there are a few inferences that can be drawn from the experimental data.

The genes seem to act continually throughout development; for as long as cell division is going on the amount of genic substance is increasing; and this, as we have seen, involves an autocatalytic activity on the part of the genes. The fact that some genes, although they multiply, seem under certain circumstances to produce no effects (as recessive genes in hybrids), suggests at first sight that the growth of the gene may be separated from its activity in bringing about development. But this is probably an incorrect interpretation. For if the gene were itself inactivated, all its effects should disappear together; whereas often some of the effects persist despite the fact that others are inhibited. It seems therefore more likely that what is inhibited is not the activity of the gene itself but that of some substance produced by it. One plausible interpretation, proposed by Goldschmidt, is that the inhibiting gene or some substance it produces reacts more quickly than a corresponding substance produced by the other gene; and that when the quickly produced substance reaches a certain concentration, the character in question is definitely determined so that it cannot be altered by the slower substance even when that becomes more concentrated. This would explain dominance as well as the inhibiting effects of genes of different pairs. But it is not the only possible explanation; and obviously not all cases of interaction

are of this kind; since often neither gene is entirely inhibited but the effect produced is intermediate.

That the genes act even in the early embryo is shown by the fact that genes introduced by the sperm may produce visible effects at very early stages of development. There is, however, evidence that they do not act in the sperm of animals; for sperm cells containing different sets of genes do not differ in their activity; and this is true ordinarily even if we compare X-bearing and Y-bearing sperm, which differ in an entire chromosome. The inactivity of the genes in the sperm must be due to the lack of appropriate cytoplasm, since the genes begin to act when they enter the egg. In plants the genes of the sperm nucleus do exercise an influence on the pollen, where they are surrounded by a considerable amount of cytoplasm.

The autocatalytic activity of the genes must continue as long as cells continue to divide and grow. But some cells (like those of the brain) cease to divide after a certain stage of development; and if the genes are active in these, the activity must be non-autocatalytic—unless the material manufactured is genic but is not incorporated into the gene.

There must be numerous intermediate processes between the activity of genes and the final formation of many of the structures we call characters. Some progress has been made in analyzing the transformations of matter and energy that go on in development, although the physiology of the embryo has been studied much less extensively than that of the adult. Attempts have been made to distinguish the activities of the gene from those of intermediate substances; but none of these attempts has been successful because no experimenter has

yet shown that he was dealing with the genes rather than with some substances produced by them.

MUTATION AND EVOLUTION

Changes in Genes.—Having admitted an exception to the rule that genes are not sorted out in development, we must now admit an exception to the rule that they remain unchanged. For although in general genes seem to be unmodified despite their activity, occasionally a gene changes or mutates. A mutation, being an alteration of a gene, may alter any or all the characters that the gene affects; it may modify them in any direction and to any extent. It may make an organism larger or smaller, darker or lighter, more or less viable, etc. Usually, to be sure, mutations result in a decrease in viability, and often even in death. But this is to be expected, for a random change in a complicated mechanism is much more likely to interfere with than to improve its functioning.

It is by the selection of (presumably beneficial) mutations that evolution has come about; for the differences between some species have been shown to be inherited in the same way as those arising by mutation —that is, in Mendelian fashion. But abnormal distributions of chromosomes and pieces of chromosomes must also have occurred, since species may differ in the number of chromosomes and in the arrangement of genes within them.

Mutations have sometimes been interpreted as losses of genes. If this were correct, a single gene could mutate in only one way—by dropping out. But this is not the case. In Drosophila one gene has mutated in some cases so as to alter both the wings and the thorax, in some

cases so as to alter only one or the other; mutations in another gene always affect principally the eye color, but the resultant colors range from white to red; and more such instances might be cited. Moreover, mutation is reversible, since some mutant genes change back to their original condition, which they could not do if they had been lost.

Mutation may occur in any cell at any stage of development, but usually in only one cell at a time and in only one gene in a cell. The fact that only one gene in a chromosome is affected, and only one gene of a pair, indicates that the disturbance producing the change is very limited in extent.

A mutant gene is usually transmitted without further change to descendant cells. If the new gene arises before the first division of the fertilized egg, it is passed on to all the cells of the body; but if it arises later, only some of the cells will receive it, and the later the mutation occurs the fewer these will be. Hence if the mutation is one which is capable of influencing the entire body (for example, if it changes the body color) the approximate stage at which it occurred can be deduced from the proportion of the body that is affected. These facts throw light on the old question whether the owl or the egg came first, or (to restate it in modern genetic terms) whether the change responsible for a new character occurs first in the mature germ cells or the somatic cells. The answer is that it may appear in either, or in a cell ancestral to both; but only if the germ cells are affected is the change passed on to the next generation. It is obvious, therefore, that a mutation is not necessarily (as it has sometimes been defined) a change that is inherited.

Rates and Causes of Mutation.—The rate of mutation varies for different genes in an organism and probably for genes in different species. In Drosophila the average length of life of a gene, as determined by Muller and Altenburg, is not less than two thousand years, a time roughly comparable to the life span of a radium atom. This value was obtained for a temperature of 26° C.; for a decrease of ten degrees in temperature the rate is about halved, as is usual in chemical reactions, and this suggests that mutation is a chemical change.

Heat may therefore be considered a cause of mutation, though perhaps not a very efficient cause. Other environmental agents have been tried but always with negative or ambiguous results until Muller showed in a series of remarkable experiments that X-rays can increase the mutation rate over a hundred times.

The question whether genes can be modified by the environment is therefore answered in the affirmative; and it is probable that other agents besides heat and X-rays will be found to be effective. Perhaps, too, it may become possible to alter a particular gene in a particular way, which at present we are unable to do.

But it does not follow that the gene is not susceptible to influences from the organism itself. Theoretically it might be modifiable by the cytoplasm or by other genes, or it might mutate spontaneously—that is, as a result of forces within itself. The last possibility would be hard to prove or disprove, since it would involve ruling out all other causes. But the other hypotheses have been tested experimentally, and in general the results have been negative.

In one experiment the ovaries of a black guinea-pig

were grafted into the body of a white guinea-pig; but despite the white environment the eggs (when fertilized by sperm from a white male) developed into black offspring. The facts of heredity show that genes may sojourn in bodies of many kinds without being altered. Segregation in hybrids proves that the genes of a pair do not affect each other's constitution, and in general this is true also for genes of different pairs.

There are, however, some indications to the contrary. For example, certain genes are known to mutate more frequently in the presence of certain others—an effect that might be produced through the cytoplasm. This suggests that the gene is susceptible to influences from all quarters and that whether or not an effect is produced depends on the strength or nature of the stimulus.

Inheritance of Acquired Characters.—The above results have a bearing on the question of the inheritance of acquired characters. Stated in modern terms, this doctrine would imply the following processes: (1) The environment must affect the cytoplasm of some somatic cells (either directly or through the genes in these cells). (2) These cytoplasmic changes must alter some gene or genes in the germ cells. (3) The altered genes in the germ cells must produce a change in the characters of the next generation. (4) The change in the characters of the next generation produced by the altered genes must be the same as the change in the cytoplasm of the first generation produced by the original environmental stimulus. In view of what has been said, it is obvious that (1) and (2) are possible, though (2) is perhaps improbable unless the change in the cytoplasm is very great; (3) would be likely to follow if (1) and

(2) were true. But (4) would represent a very remarkable coincidence; there is no known reason why the changes in the two generations, which are due to different causes, should be the same: and it seems much more probable that they would in general be different. The result of this analysis is supported by the fact that all crucial experiments designed to test the inheritance of acquired characters have given negative results.

Mutation and Development.—Although mutations are not responsible for development, they may affect its course. It has been suggested that cancer is due to mutations in the cells of the body; that a person susceptible to cancer inherits some genes which, while they do not themselves produce the disease, are comparatively unstable and likely to change, so that the cells containing them become cancerous tissue. According to this interpretation, such mutations do not occur in a noncancerous individual or else occur so infrequently that no appreciable effect is produced in a lifetime. It is also possible that apart from cancer, old age and death are due to the accumulation of mutations in somatic cells; for as mutations are random changes in a complicated mechanism, they are likely to be deleterious. At present this is merely conjecture. Genes may be too stable for such an effect to become appreciable in a lifetime, and death may be due to developmental processes proper. But the occurrence of mutations suggests the impossibility of prolonging the life of an individual indefinitely. For if all the genes are mutable even to only a slight extent, only a finite (though perhaps a very long) time is required for the accumulation of a sufficient number of deleterious mutations to produce death.

HEREDITY AND ENVIRONMENT

The demonstration that characters depend on heredity has so impressed some biologists (and many who are not biologists) that they have jumped to the conclusion that environment is of no importance. Such a view is not in accord with the facts of development, for every character depends on both genes and environmental influences. To ask whether heredity or environment is the more important is like asking whether the area of a rectangle is more dependent on its base or its altitude. Or as Muller has put it, the fallacy implicit in the question is the assumption that the character may be represented as the sum of heredity and environment; whereas it should be considered as some non-linear function like the product.

It is obviously impossible in general to compare heredity and environment by expressing them in common units. Some statisticians have therefore sought to avoid the difficulty by measuring the extent to which variation of a character is correlated with its variation in parents and with differences in the environment. There are, however, two difficulties with this method. In the first place, the correlation between traits in parents and offspring is not necessarily a measure of the strength of heredity: the correlation between the language of father and son would be very high, but it is due to similarity of environment. In the second place the result holds only for the range of heredity and environment studied: even if it be demonstrated that existing differences in environment do not alter a character more than a certain amount, it does not follow that the character cannot be altered further by other environmental differences. Be-

fore the antitoxin treatment of diphtheria was discovered it would have been correct to say that no environmental agent was known to be capable of preventing the disease; but it would have been fallacious to infer that diphtheria could not be prevented by environmental agents as yet unknown, and to conclude that only by breeding for a hereditary immunity could it be stamped out.

The argument can, of course, be reversed. The fact that a particular effect has not been brought about by mutations or recombinations of genes does not enable us (in our present state of knowledge) to predict that its occurrence as a result of such changes is impossible. The realization of this has given rise to eugenics, which aims to improve the qualities of mankind by altering its heredity. This might be done by selecting mutations that have desirable effects, or by making new combinations of genes already in existence. For accuracy in such a procedure a far greater knowledge of human heredity is necessary than we have to-day; but doubtless our descendants will ultimately possess this knowledge. Perhaps a greater difficulty in the way of a eugenic program is the great diversity of opinion as to what qualities are desirable; unfortunately most persons who call themselves eugenists have attempted to evade the difficulty by setting up their own prejudices as standards. But for those qualities with which the physician is concerned this is not a great obstacle, since even now it is possible to decide objectively what constitutes good health.

THE GENES (THEMSELVES)

We have spoken of the number and size of the genes, of their location and arrangement in the chromosomes, of their interaction and mutation. But what are the

genes themselves? A discussion of this question is likely to resemble the conversation between the passer-by and the small boy who was fishing through a grating. "What are you fishing for, my little man?" "Wooglies." "Wooglies? What are they?" "Don't know; haven't caught any yet." The geneticist must admit that he has not yet caught any genes in the sense of isolating them and subjecting them to chemical analysis.

It is true that some information can be obtained by analyzing sperm, which contains very little besides chromosomes. Such investigations indicate that chromatin, although it varies in different species (fishes and echinoderms have been studied) consists of a salt of a nucleic acid and a protein base. But these results do not necessarily represent the constitution of individual genes. For in the first place the sperm, while it consists largely of chromatin, does contain a small amount of cytoplasm. In the second place the chromosomes themselves may not be made up entirely of genes—the Y-chromosome may carry no genes or almost none. And in the third place, the sperm is a mixture of genes, and the methods employed in making the chemical analysis may be incapable of distinguishing between the different kinds and may therefore represent only an average or mass result.

Microchemical methods suggest themselves in this connection. But the gene, which is below the limits of visibility, is far smaller than the minimum mass required by even the most refined microchemical technique. Perhaps some method like positive ray analysis, for which only very minute amounts of matter are required, may make it possible to detect differences between sperm of mutant races and to obtain information

about the chemical composition of the genes in which they differ. If the geneticist of the future can thus analyze organisms directly, the breeding that must be carried on in present day experiments will seem to him as cumbrous as it will be unnecessary.

Mutations produced by X-rays or other environmental agents may give us a clue to the structure of the genes, as the breaking up of molecules and atoms has served to elucidate their composition. Since every kind of atom absorbs X-rays of a characteristic wave length, homogeneous rays of various wave lengths may, by affecting atoms of different kinds, produce mutations in different genes or different mutations in the same gene, and so enable us to study their chemical constitution.

The chemical constitution of the gene brings up the question whether it is a single molecule or composed of several molecules. So far as its size is concerned it might be a single large molecule. There is some evidence that the gene is composed of simpler units (genomeres) because some experimental results can be interpreted on the supposition that the parts into which a gene divides are not always identical. But it is possible that the parts are alike when formed and that subsequently one of them mutates. Hence the genomere interpretation must be regarded as only provisional.

Although the biologist has not yet reduced his units to those of chemistry and physics, he has analyzed the phenomena of heredity and development in terms of matter and its configurations. All the indications are that it is in this direction that an explanation of life is to be sought. In the past, heredity and development were obstacles to the acceptance of a mechanistic interpretation of life. The development of a complex organism from an egg seemed inexplicable as long as the egg

was looked upon as a homogeneous body in a uniform environment. The problem resembled that which confronted the Greek philosopher Parmenides who, regarding reality as a homogeneous sphere, found himself unable to reconcile it with the heterogeneous universe of his senses. He decided therefore that the sphere represented the way of truth and the universe merely the way of opinion. But whatever may be true of the sphere of Parmenides, the egg is not homogeneous and its environment is not uniform; and although its development into a complex organism is still very incompletely understood, it no longer seems like a contradiction in terms. The shuffling of traits in heredity at one time seemed mysterious; but with the elaboration of the Mendelian theory the problem of heredity has been solved. The biologist has shown that what is inherited is composed of genes located in the chromosomes; and he has mapped their positions and determined their modes of transmission from generation to generation.

The nature of the genes and how they interact to produce the characters are still unsolved problems. The genes have hitherto occupied a position of splendid isolation: they directed the processes in the cell and yet seemed themselves entirely unaffected by what went on about them. They seemed eternal and (apart from rare mutations) changeless; and even when they changed they appeared to do so because of forces within themselves and not in response to external stimuli. Now, however, that it is known that mutations can be caused by the environment, the isolation of the gene is seen to be illusory; and we may hope, by studying its changes under various conditions, to learn something about its nature and how it produces its results.

CHAPTER III

The Human Body As A Machine

Article 1

THE BODY AS A CHEMICAL ENGINE

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Dr. Murlin held important appointments in such leading medical schools as the University and Bellevue Hospital Medical College of New York (1903-9) and the Cornell University Medical College (1909-17) before taking up his present important work at the University of Rochester. In 1917-19 he was Lieutenant-Colonel of the Sanitary Corps and Director of Division of Food and Nutrition in the Medical Department of the U. S. Army. He has made eminent contributions in the field of nutrition and metabolism, notably metabolism in infancy, pregnancy and diabetes.

JUST over fifty years ago Huxley came to America on invitation to deliver the opening address at Johns Hopkins University. Standing at the rail of the steamship which brought him into New York harbor, and observing the power and agility displayed by the tugboats which aided in warping his ship into the dock, he remarked, "Well, if I weren't a man, I think I'd like to be a tug." Huxley was always impressed with the resemblance between the human body and an engine and his remark implied, to one who knew his teaching, that the alteration required would not be so great as if he were to change, for example, to a house. A tugboat is all power and can apply its power in such

a variety of ways as to appear almost alive. In his short autobiography Huxley tells us that it was his ambition as a boy to be a mechanical engineer, but the fates were against this and he began very early to study medicine under a medical brother-in-law. He confesses that the only part of his professional course which really interested him was physiology, which he calls "the mechanical engineering of our human machines." Today we should add "chemical engineering." This chapter will deal with only a few of the mechanical and chemical engineering problems of the human machine.

The comparisons commonly made between the body and an engine refer particularly to the development of power from fuel. Food is fuel (and repair material, too). We put it in the stomach, digest it, absorb it, burn it, blow off the products of combustion through the lungs and so get our power. Huxley expressed this relationship as well as it could be done at that time, in 1866: "The oxidation of the complex compounds which enter the body is definitely proportional to the amount of energy which the body gives out, just in the same way as the amount of work to be got out of a steam engine, and the amount of heat from its furnace, bear a strict proportion to its consumption of fuel."

But the analogy can be carried much further than this today. Most of the engines with which we are familiar are heat engines. They accomplish work (this being the business of an engine) by letting down heat from a higher to a lower temperature just as a water wheel can accomplish work by letting down water from a higher to a lower level. To be sure, some of the heat does not arrive at the lower level because it takes the form of work and thereafter may, when the work has

been accomplished, return to the form of heat, but outside the engine. From the point of view of practical thermodynamics the object of the heat engine is to get as much work done for a given expenditure of fuel energy as possible. The ratio of work done to the total heat taken in by the engine, whether as steam or as gases from internal combustion, expressed in the same units, is called the thermal efficiency of the engine. Since Joule in 1843 worked out the mechanical equivalent of heat, or the heat equivalent of work, we can express the work of an engine or of the body and its available heat in either of these units—calories or B. T. U.'s as units of heat, kilogram-meters or foot-pounds as units of work. Joule found that 772 foot-pounds of work will raise the temperature of one pound of water one degree. (This is the British Thermal Unit, or B. T. U.) The accepted figure today is 777.8 foot-pounds. In the metric scale the equivalent of a large Calorie is 426.7 kilogram-meters. In other words, if we could transform all of the fuel energy of coal into work, every B. T. U. would raise 778 pounds of weight one foot high, or every Calorie of our food (if we could convert its potential energy entirely to work) would enable us to raise a kilogram of body weight 426.7 meters high. This would be 100 per cent thermal efficiency. As a matter of fact all engines, including the human engine, fall far below this ideal.

THE EFFICIENCY OF AN ENGINE

What is it that determines the efficiency of an engine, say of an automobile? An answer to this question will give us points of view which will be of value in answering the same question for the body. So far as the first

law of thermodynamics, otherwise called the principle of the conservation of energy, goes there is no reason why all the energy imparted to the expanding gases in an automobile cylinder should not take the form of mechanical work. But the second law, called also the principle of Carnot and Clausius, sets a limit to the first law. It states in one of its simplest forms that free energy always tends to a minimum; or, conversely stated, run-down energy, called entropy by the engineer, tends to a maximum. As applied to the heat engine it states merely that heat will not pass automatically from a cold body to a warmer one, but only in the reverse direction. It is only by letting heat down from the high level imparted to the gases by combustion to the lower level of the exhaust that work is done. The high temperature causes expansion of the gases, thereby pushing the piston; the lower level causes contraction and the pressure is greater during the former than the latter. As a consequence a part of the heat is converted to work.

Sadi Carnot, the Frenchman, whose name was just now applied to the second law of thermodynamics, in 1824, twenty years before the law of the conservation of energy was formulated, pointed out that the greatest possible amount of work could be obtained from an engine by letting the heat pass from its source to its outlet—in the steam engine this would be from the inlet into the cylinder from the boiler to the outlet from the cylinder to the condenser (or merely to the outside air)—through the engine working in a strictly reversible manner. This is Carnot's ideal cycle. It includes four phases. Referring to the figure, the four

phases are indicated by the volume-pressure relations ab, bc, cd, da.

The hot body A, the insulator B and the cold body C can be applied successively to the cylinder, which is filled with a perfect gas. (1) Apply hot body A; the gas expands and drives the piston without warming the gas, i. e., it expands isothermally. The pressure changes to Pb and the volume to Vb. (2) Now remove A and apply B (this is more than equivalent to closing an intake valve). The piston will go on advancing for a time, but now that the source of heat is withdrawn, the gas begins to cool. As a matter of fact it cools because it expands. No heat is lost from the cylinder as

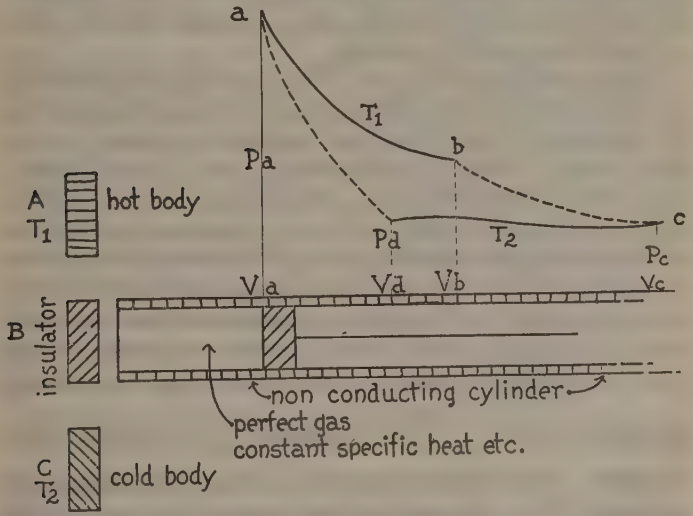


Diagram of an ideal engine illustrating Carnot's cycle of four phases. (After Sir James Alfred Ewing in "Dictionary of Applied Physics," 1922, Vol. 1, p. 932.)

such, and this part of the expansion is called adiabatic for this reason. (3) Now remove B and apply C. This is the cold body, and if the piston is now forced back (by the stroke of another cylinder) the gas will be compressed without rise in temperature, i. e., isothermally, since the slightest increase of temperature above T_2 will cause heat to pass into C. Work is spent on the gas and heat is rejected into the cold body. Let this go on until a certain point d is reached on the back stroke, when (4) C is removed and B is once more placed over the cylinder. The compression goes on, but now the pressure will rise rapidly and the temperature likewise. If the point d has been properly chosen the pressure Pa will be reached at the same moment that T, the original heat level, is attained.

This completes the cycle. The criterion of perfection or of efficiency is the completeness of the reversibility. A completely reversible heat engine is perfect in the sense that it cannot be improved upon for efficiency. No other engine taking in and giving out heat at the same two temperatures can obtain a greater proportion of the heat as mechanical work. Mathematically it can be shown that the net amount of heat converted to work is the excess of the heat taken in above the heat rejected, and the efficiency of the cycle, namely, the ratio of the heat converted to work, to the initial heat, can never be greater than $\frac{T_1 - T_2}{T_1}$, expressed in absolute temperature. Hence this expression measures the maximum efficiency of any reversible heat engine. As a matter of fact no real engine can attain this efficiency, because of inevitable losses of heat.

IS THE BODY A HEAT ENGINE?

In a steam engine or internal combustion engine there intervenes between the chemical change (combustion) and the mechanical effect a thermal stage in which the whole of the energy contained in the fuel becomes kinetic as heat. Does this happen in the animal engine, or is the chemical energy transformed directly to physiological energy? There is plenty of heat produced in the body, particularly in warm-blooded animals, and it is a familiar fact that the greater the physical work done by the body the greater is the production of heat. Does this heat production precede the performance of mechanical work, or does it follow? It is interesting to note that J. R. Mayer, who, coincidently with Helmholtz, discovered the principle of the conservation of energy in 1845, and who indeed was led to reflect upon these matters from consideration of the performance of muscular work in man, thought that the muscle engine is a heat engine. Engelmann also thought so, and worked out a little model of a muscle fiber, represented by a catgut violin string, which could be made to shorten by imbibition of water from a bath in which it was immersed upon being heated momentarily by means of a fine coil of platinum wire wound about the string. But the German physiologist Fick in 1882 clearly stated the objections to such a view. Fick was familiar with the Carnot cycle and with the principle resulting from it, that the work accomplished by a heat engine depends upon the difference in absolute temperatures between the intake and exhaust. Fick used some interesting examples from his own experiments with frog's muscle to show that the amount of work which it can per-

form is so great in proportion to its mass, that to account for this work-quotient on the Carnot principle would require a change in temperature quite incompatible with life. His conclusion was that the performance of work in a muscle and *à fortiori* in other tissues of the body is not a thermodynamic process. He makes the statement that the "chemical forces" of the body in some way are used directly for the performance of mechanical work. Chemical energy we know can very readily be transmuted into other forms without the necessity of passing through the form of heat. For example, in the primary electric battery (not the storage battery) the current is derived directly from chemical reactions and can be used to drive motors, but no heat or very little appears until after the work is done.

But is there not some special form of energy, call it "vital energy" or "biotic energy," which is peculiar to living organisms—something quite different from ordinary chemical and physical energy? We cannot here enter into an extended discussion of this philosophically interesting question of vitalism. From the standpoint of the physiologist the answer must be that no instrument has ever been devised which could detect or measure any such form of energy as, for example, the electrometer measures electrical potential or a thermometer measures heat; and until such a form of energy can be rendered evident to some one of the human senses it must be treated as non-existent. It may be noted in passing, however, that, as Benjamin Moore has stated, "it is in the transfer of energy from one kind to another that the activities which are peculiar to organisms exhibit themselves." A static system is dead; a living thing is peculiarly dynamic.

FUEL OF THE MUSCLE ENGINE

In following up the modern conception of the muscle engine, our first inquiry will be regarding the nature of its fuel. Can the muscle be driven by any kind of fuel, as the steam engine can be, or must it have a specific kind of fuel like the automobile?

It is now possible to measure the heat of contraction of a single frog's muscle to a high degree of accuracy and to compare it with chemical changes in the muscle. Chiefly through the work of A. V. Hill, now Foulerton Professor of Physiology of the Royal Society of Great Britain, and his pupils, of whom Dr. W. O. Fenn is one, thermo-electric devices have been prepared which will detect a change in temperature of 0.00001° C. in a muscle removed from the body and suspended in a small chamber where it can be stimulated at will. Chiefly through the work of Fletcher and Hopkins in England and of Meyerhof and Embden in Germany the chemical changes which could produce the energy appearing as heat have been closely analyzed.* Hill discovered that the heat liberated in a single muscular twitch, following a single induction shock applied directly as a stimulus, appeared in two nearly equal portions: the first appears during the contraction process and is called the initial heat; the second appears during the recovery process and is called the delayed or recovery heat. When the muscle is suspended in an atmosphere of nitrogen only the first or initial heat appears, showing that the chemical process supplying the heat is not oxidation, showing indeed that oxygen

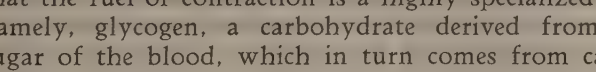
*Hill and Meyerhof received jointly the Nobel prize for 1922 in recognition of their outstanding contributions in this field.

is not necessary in this process in any manner—that the chemical change, in short, is anærobic. The muscle engine does not depend on oxidation, as do the various forms of heat engines with which we are familiar, for the immediate release of energy. For the recovery process, however, getting ready for the next contraction, oxygen is essential. Combustion takes place, and as the result the muscle is wound up, so to speak, as the spring of a watch is wound up, ready for the next stimulus which will trip the spring and bring on a contraction capable of doing work.

It was Fletcher who first observed that in an atmosphere free of oxygen *lactic acid* accumulates in a muscle even when it is resting—still more when it is made to contract; while in an atmosphere of oxygen the lactic acid disappears. This led to the theory formulated by Hopkins and Fletcher, that some precursor of lactic acid contains the initial energy and that it is released by the change to lactic acid. The weight of evidence, furnished chiefly by Meyerhof, is that glycogen, or animal starch, is this precursor. Its formula is $(C_6H_{10}O_5)\,n$. By absorption of water it becomes $C_6H_{12}O_6$ and by direct change it can form two molecules of lactic acid, $C_3H_6O_3$. Now the heat value of a gram of hydrated glycogen is 3,836 small calories per gram; of lactic acid in solution 3,601 calories. The difference, or 235 calories, is the heat liberated in the initial or contraction phase by the change from one to the other. In addition 135 calories are given off in the subsequent neutralization of one gram of lactic acid by the alkalinity of the blood. The total, or 370 calories, approximates very closely the amount found by Meyerhof for each gram of lactic acid which could be recovered.

It should be clearly understood that the heat which accompanies the contraction of muscle is in no respect comparable with the heat transferred in the form of expanding steam to the cylinder head of a steam engine, or more directly by expanding gases of combustion in the internal combustion engine. The heat in the former instance represents energy liberated by the chemical change which has first taken the form of surface tension or osmotic pressure before it can be detected by the thermo-electric instrument. These aspects of muscular contraction, in many respects the most interesting of all, are not yet fully elucidated and therefore need not detain us. Enough has been said to demonstrate one of the essential differences between a heat engine and the living muscular engine. There are some points of resemblance which will be emphasized later. For the present—and this gets back to our question—we note that the fuel of contraction is a highly specialized one: namely, glycogen, a carbohydrate derived from the sugar of the blood, which in turn comes from carbohydrate or protein of the food. The end product of contraction is lactic acid, merely a simpler combination of carbon, hydrogen and oxygen than we started with.*

* *Editor's Note.*—The change of glucose, formed from glycogen, into lactic acid



is the result of a *shifting of electrons* from certain carbon atoms to other carbon atoms with a consequent shift of H and O atoms. It no doubt represents a fall of energy to a lower level. This release of energy requires no outside oxygen, but probably is stimulated by the alkalinity of the blood, since the same change has been observed as the result of the action of weak alkali on glucose.

Now the question arises what becomes of this lactic acid? If it were completely oxidized as Fletcher and Hopkins originally supposed, we should get as the net result the full heat value of glycogen, namely, 3,836 calories per gram; whereas we actually get in the contraction phase less than one-tenth this amount.

The heat of the recovery phase also can be measured in the excised muscle, for while in the living frog the recovery takes place almost instantaneously, in the muscle removed from its blood supply recovery requires two or three minutes. This heat of recovery turns out to be slightly larger than the heat liberated in the initial phase, and, as we have learned, is dependent upon oxygen.

Meyerhof observed that the relation of carbon dioxide formed in the recovery process to the oxygen used (a ratio called the respiratory quotient) is such as to indicate that carbohydrate is the fuel burned; and since the lactic acid which was formed during contraction has now disappeared, it is practically certain that lactic acid is this carbohydrate fuel. The heat production of the combustion, however, is again only one-tenth what it would be if the full quantity which appears in contraction were burned up. The total heat of contraction and recovery is approximately one-fifth that which would be formed if all the glycogen were burned. It appears, therefore, that the balance of the lactic acid, instead of being consumed, is resynthesized to glycogen, and indeed this is the purpose of the oxidation taking place during recovery—namely, to furnish the energy for rewinding the spring or, as Hill expresses it, for *recharging* the battery.

At all events the mechanism is brought back to the

condition from which it started after each contraction, unless the contractions are made to succeed each other at too rapid a rate, when, as everybody knows, fatigue sets in.

This restoration to the original condition suggests the reversibility of the heat engine which Carnot made the criterion of efficiency. Can a similar criterion be applied to the muscle engine? It becomes steadily clearer that the muscle engine resembles an electric battery much more than it resembles a steam engine, and it need not surprise us to learn that different criteria must be applied to the body whether it be considered in its entirety or organ by organ. Nevertheless, there is a sense in which this matter of reversibility to the original state becomes a test of efficiency for muscle (and probably for many other forms of physiological work). The energy required to put the piston back into position (a) the moment at which temperature and pressure are highest is a large factor in determining the actual rather than the theoretical efficiency of a heat engine. Likewise the amount of combustion necessary to build up again the lactic acid to glycogen is a large factor in making up the efficiency of muscle. The glycogen re-formed from the lactic acid produced in contraction never can quite equal the original charge of this material, any more than the piston can automatically return to its original position, the difference being due to the lactic acid which must be oxidized to furnish the energy for recovery. The ratio of glycogen recovered to glycogen destroyed by oxidation in the frog's muscle is about 5.2:1. Hill and Long have shown recently that the same ratio applies to human muscles. They estimated the carbon dioxide retained after the reaction of the blood had become con-

stant following severe muscular exercise, thereby securing a measure of the total lactic acid produced, and compared this with the total excess oxygen absorbed during and immediately following the exercise, which amount, assuming that nothing else than lactic acid is oxidized, can be made a measure of this oxidation. This ratio, however, has nothing to do with the mechanical efficiency of muscle, which will be considered presently.

Meantime, a very pretty controversy has developed over the question of whether carbohydrate only can be oxidized to restore lactic acid to glycogen, or whether fat can be so used by the animal engine. As a matter of fact the controversy is older than the exact matter controverted. For Cheauveau, long before the Hill-Meyerhof conception had been developed, made the assertion that only carbohydrate is burned in rapid muscular work, such as running up and down stairs, and he backed up this assertion by pointing out that when fat is used to support muscular work there is a 30 per cent wastage owing to the necessity of first converting it to sugar before it can be fed into the fire. It is comparable with the wastage which would be involved in distilling coal to get a fuel which could be fed into an automobile through the carburetor.

Zuntz very promptly took up the gauntlet thrown down by Cheauveau and produced evidence which seemed conclusive, from experiments on horses, dogs and humans, that fat can be used just as well as carbohydrate in supporting muscular work, and that the animal engine has such powers of adaptability that whether one fuel or another is available makes no material difference in the efficiency with which potential or fuel energy of its food can be put into horse-power, dog-

power, or man-power. Here the matter rested from about 1895 until 1920, when two Danish investigators, Krogh and Lindhard, reopened the question. They were studying the relative efficiency of the human muscles employed in bicycle riding, when on the one hand the subject was maintained on a nearly exclusively fat diet and on the other on an exclusively carbohydrate diet. The result was a difference of about 10 per cent in favor of the carbohydrate diet. Their interpretation of the result is that the loss of 10 per cent when fat is burned is due directly to the necessity of its conversion to carbohydrate. As Hill and Meyerhof would express it, the *recovery process* is 10 per cent more efficient when carbohydrate is oxidized to restore lactic acid to glycogen than when fat is so oxidized, and Hill argues, as did Cheauveau, that because carbohydrate oxidation is more efficient "fat is used in only a secondary manner to supply the carbohydrate which has disappeared."

Lusk challenged Hill and Meyerhof to produce any independent evidence that fat can be changed to carbohydrate in the animal body. His conception is identical with that of Zuntz, and he emphasizes the fact that in diabetes, under circumstances when all the carbohydrate fed appears in the urine as sugar, the feeding of fat never increases the sugar output more than can be accounted for by the glycerin contained in the fat. If this change to carbohydrate were so readily accomplished whenever sugar ran short in heavy work, it would seem that muscular effort in severe diabetes where no sugar can be burned would increase the excretion of sugar. Fasting should do the same. But as a matter of fact both tend to diminish the excretion.

Some of Hill's pupils recently have brought forward

evidence from short running-in-place experiments that carbohydrate furnishes the only source of energy in this type of exercise. The subject while running breathes into a large air-tight bag. When this expired air is analyzed and its composition compared with that of the air which entered the subject's lungs, the respiratory quotient indicates combustion of carbohydrate for the extra energy of running as compared with the energy output while standing still. Experiments carried out by Miss Marsh in our own laboratory on the muscular efficiency of boys do not bear out this conclusion. As a matter of fact the respiratory quotient for short spurts of work indicates the combustion of whatever foodstuff is predominant in the alimentary tract or recently acquired by the circulation at the time. These are in agreement with Henderson's observations on the Yale crew while rowing the four-mile course a couple of years ago. Hill in some of his latest papers appears to agree that except for very short spurts other foodstuffs than carbohydrate may *support* the recovery process, but he would hold that all must become carbohydrate before actual combustion. Here we must leave the controversy until somebody hits upon a perfectly crucial experiment.

THE "OXYGEN DEBT" AND SUPERIORITY OF THE ANIMAL ENGINE

All experiments in this field must now take account of what Hill calls the "oxygen debt." Mention must be made of it here because it illustrates one other point of superiority of the animal engine over a man-made engine of any sort. Every driver of an automobile knows how small a thing may interfere, even to the

point of fatality, with the operation of his carburetor, how quickly the mechanism dies the moment combustion ceases. There is no reserve of energy stored in the springs to get you home when the tank runs dry. Nor can the engine run you home tonight and make up for the expenditure by panting till morning in the barn, as the old bay mare could do when horses furnished the motive power. In other words, however much the owner may owe on the car the engine must pay as it goes. But the animal engine can go into debt for its oxygen. A track man can run his hundred yards in ten seconds by breakdown of glycogen to lactic acid, and spend the next half hour getting oxygen enough to burn up the lactic acid which has accumulated in his blood and tissues, thereby recharging his muscles with glycogen for the "quarter mile" or whatever is the next event. No observation on this type of experiment is complete until the debt has been paid. Hill has shown that it may require as much as seventy minutes to pay the last cubic centimeter of oxygen after work equivalent to that of a hundred-yard dash. Efficiency in the sense of paying up promptly and getting ready for the next race depends upon training, which means on the one hand doing the job with the least production of acid and on the other a developed capacity for removing or neutralizing the acid.

With the oxygen debt cancelled and the total cost in oxygen absorption therefore available one may then calculate the total energy expenditure of the body over and above the resting metabolism occasioned by any piece of work. Expressing both the work and the cost in similar terms, the mechanical efficiency is the per-

centage of cost which the work accomplished represents. The analogy with the heat engine is obvious. The human machine, moreover, compares quite favorably in efficiency with man-made engines. It is a remarkably good steam locomotive which can convert 10 per cent of its fuel energy into mechanical pull upon the train of cars. Internal combustion engines do much better, reaching 20 per cent in the best automobile engines and 40 per cent in the Diesel steamship engine.

In the body efficiency depends on several factors; as type of work performed, training, amount of load, rate of work, etc. The leg muscles are more efficient ordinarily than the arm muscles. A trained mountaineer is much more efficient in climbing mountains than a tenderfoot. A trained bicycle rider gives a higher efficiency than an untrained person on the same wheel. A moderate load and a moderate speed give higher efficiencies than the extreme in either case. The highest figures obtained on men are roughly the same for expert bicycle riding and expert mountain climbing; namely, about 33 per cent. For ordinary bicycling an efficiency of 23 per cent is considered very good; this is for adults. For boys 14-16 years old Miss Marsh obtains efficiencies two or three per cent lower than this, and for a short-legged boy who fitted the bicycle well it was 1 per cent higher than for a very tall boy with too much leg. Some experiments done in our laboratory two years ago in which the arm muscles were used to lift weights over pulleys gave efficiencies of around 16 per cent. Recently Mr. Morrison and Dr. Gaebler found slightly higher efficiencies on cereal diets than on a meat diet, the difference being traceable to the so-called specific dynamic effect of the high protein of meat to increase the heat produc-

tion independently and therefore to increase the total cost of the work. Dr. Fenn in his work with Hill was able to measure separately the anærobic efficiency of the frog's muscle and to deduce the oxidative efficiency. The former was 25 to 30 per cent and the latter 10 to 12 per cent at most. An interesting phase of these experiments was the rediscovery that a muscle cut out from the body *almost thinks*. Heidenhain had observed in 1864 that the adaptation of effort on the part of an excised muscle to the load which it must lift is, as he expressed it, "to a certain extent the function of the muscle itself." Somehow this observation had escaped the text-book makers, or else was regarded by them as quite fantastic. Fenn independently observed the same phenomenon, which he describes in these words: "The energy liberated by the contraction of a single muscle fiber for a given stimulus is not dependent solely upon the initial mechanical and physiological condition of the muscle, but can be modified by the nature of the load which the muscle discovers it must lift after the stimulus is over." There are therefore two mechanisms in operation to adjust the effort to the work our muscles have to do. One is nervous, calling more or fewer fibers into action according to need. "The other is independent of the nervous system and works by virtue of the fundamental nature of the muscle machine, whatever that may be." The two operate somewhat as the coarse and fine adjustments of the microscope work to bring an image into view. The self-adjusting fiber is the finer mechanism, instantaneously active but capable of producing only relatively small variations. Thus again is the physiologist baffled in trying to reduce vital phenomena to purely physical rules of conduct. The single

muscle fiber somehow senses its job and adapts itself accordingly. The whole organism can do no better.

CHEMICAL ASPECTS OF THE LIVING ENGINE

Thus far the physical features of the living engine principally have been stressed. Even a heat engine, however, is in a sense a chemical engine. Still more so, as we have seen, is the muscle engine. When attention is directed to the source of energy in a muscle and more convincingly in the case of other cells which do work, or even those which do no external work but still make use of energy, it is seen that the mechanism is chemical rather than physical. However, the border land between chemistry and physics is here quite obscure. The new subdivision called physical chemistry and the still newer one called bio-physics illustrate the necessity of reaching over or tearing down fences between different cultures in the pursuit of knowledge.

Time does not permit a very extended discussion of the chemical aspects of the living engine. Perhaps the question of greatest interest to physiologists is this: How does it happen that the fuel, which outside the body must be heated to several hundred degrees of temperature to make it burn, becomes oxidized in the body so as to yield up all its energy within a few hours without more than a very gentle warming? There are two principal theories: One that somehow oxygen when taken into the body gets activated by some catalyzer so that it is more active, has more "zip;" or, more learnedly spoken, has free chemical bonds, which in atmospheric oxygen are satisfied by each other. The other theory is that the food itself undergoes activation likewise by

catalysis and therefore is more eager for combustion, so to speak, than ordinary materials. There are many adherents of these two views. Chief spokesman for the former in recent years has been Warburg at Berlin. Chief advocate for the latter is Sir Gowland Hopkins at Cambridge, although the theory itself goes sometimes by Wieland's, sometimes by Thunberg's name.

THEORY OF OXYGEN ACTIVATION; FERTILIZATION AND OXIDATION

Warburg is able to cite such experiments as the following: When sea-urchin eggs are fertilized artificially, as by exposure to valerianic acid in sea water, the absorption of oxygen increases as much as eight- or ten-fold. All substances which cause artificial parthenogenesis, as Loeb calls this phenomenon, in like manner though not always in like quantity, stimulate the cell respiration. Shearer at Cambridge has shown further that following the natural fertilization of the same eggs the increase in the rate of oxidation may be as much as eighty-fold, and that the stimulus may result from mere contact with the sperm cell as well as from actual union with the cell. Now when sea-urchin eggs are disintegrated, as by rubbing them up with sand or by vigorous shaking, the oxygen consumption does not change if the eggs are taken previous to fertilization, but if taken immediately after fertilization the oxygen consumption falls off rapidly, showing that some structure essential to more rapid respiration had been formed by fertilization and destroyed again by disintegration of the cells. The cell pulp which results from disintegration of these eggs consists for the most part of fine granules which may be removed by rapid rotation of

the eggs in centrifuge tubes. The granules settle to the bottom and it is found that respiration still goes on in these granules. Still further, Warburg and Meyerhof have shown that oxidation in the sea-urchin egg is dependent upon small quantities of iron. Each gram of dry substance of the egg contains about 0.01 milligram of iron. If an equal quantity of iron be added in the form of a solution of iron salt, the rate of oxidation is doubled. Contrariwise, the addition of a little cyanide of potassium instantly stops respiration in these live cells, as it does, generally speaking, in all living structures, and Warburg and his school believe that this inhibition is explained by the union of the iron with the cyanide. Furthermore, narcotics inhibit the increase in respiration which is caused by the addition of iron salts.

THE IMPORTANCE OF CELL STRUCTURE

One further link in the theory supported by Warburg concerns the importance of cell structure. Every cell has a characteristic microscopic structure depending upon the kind of work it has to do. Such structure, whatever its exact nature, gives a chance for the chemical energy set free to produce an effect. In the case of voluntary muscle cells, or fibers as we call them, the characteristic cross striations in Warburg's view afford points or surfaces for the application of this free energy in a manner remotely akin to the application of an expanding gas to the piston of a cylinder. With the disintegration of this structure the utilization of such free energy is no longer possible. Other cells, such as the secreting cells of the kidney, liver, pancreas, etc., have in each case a structure which can be recognized by the cytologist as

characteristic of glands, although each is slightly different from the others. In some way not at all understood this structure, according to Warburg, furnishes surfaces of application for the free chemical energy so that it is transformed into the energy of secretion. It can be easily shown that this energy of secretion is very considerable. By putting a small glass tube into the duct of the salivary gland, connecting it with a blood pressure machine, and then stimulating the nerve which innervates the gland, a secretion pressure of saliva may be produced which is two or three times as great as the blood pressure.

Cell structure, in other words, includes all forms of structure on which any kind of cell work may be done. We might say, therefore, that the cell is intact if it is capable of doing some kind of work, and we might use as a criterion of disintegration that state of a cell in which the mechanism for doing work is lost. Some cells apparently have no capacity for converting chemical energy into a measurable form of external work. Nevertheless these cells do respire oxygen and produce carbon dioxide and heat. Why should such cells require oxygen if they do no work? Warburg's answer to this question would be that merely to maintain a structure requires an expenditure of energy through oxidation. The surfaces of cell structure afford places for condensation of iron, and such iron finely divided and exposed to a large surface serves to activate the oxygen. This is Warburg's complete theory of cell respiration.

THEORY OF THE ACTIVATION OF HYDROGEN IN THE BODY

The alternative theory proposed in its broadest form by Wieland was based upon a study by this author of

the oxidation of aldehydes under the agency of the so-called Schardinger enzyme of milk. The products obtained in the presence of oxygen were compared with those arising under the strictest of anaerobic conditions, the oxidizing agent being in the latter case methylene blue. The products were the same in both cases and Wieland was led to his fundamental conception, namely, that the enzyme obtained from milk activates two hydrogen atoms so as to make them capable of uniting either with oxygen from the air or with methylene blue. There are many substances besides the aldehydes which are capable of oxidation under the influence of living tissue, which is best explained by this theory. The withdrawal of hydrogen from a substance is to the chemist virtually the same thing as adding oxygen to it. That is to say, both are called oxidation.* Succinic acid is a substance which under ordinary circumstances is extremely resistant to oxidation. Thunberg at Lund, Sweden, made an exhaustive study of the oxidation of this material under the influence of living tissues and found that two of the hydrogen atoms are easily mobilized and are thereby prepared for union either with oxygen of the air or with methylene blue. Another instance of oxidation by this method appears to be the formation of uric acid from the so-called purin bases. This oxidation is very difficult to secure in the laboratory. Sir Gowland Hopkins states that in his belief it has never been accomplished. Yet with certain animal tissues it is very readily demonstrated *in vitro*. The agency of the tissues

* *Editor's Note.*—The loss of hydrogen or the gain of oxygen is the result of the more fundamental loss of electrons by the atoms that are oxidized. Either is therefore a sequel of the same fundamental change.

responsible for the reaction is an enzymic oxidase which also may be obtained from cow's milk. Adding a little xanthin, one of the purin bases, to an extract of cow's milk and incubating for a short time, one can witness an exactly quantitative conversion to uric acid. The only reasonable explanation of this rapid oxidation, according to Hopkins, proceeds along the lines of Wieland's hypothesis. Dixon has obtained this particular oxidase from milk in highly concentrated form and has found more than twenty substances, many of them dyestuffs like methylene blue, which can serve as hydrogen receptors for the oxidation of bases to uric acid. Dixon has also shown that this reaction is not affected by cyanides and its activity is not increased by the addition of iron. Thunberg has discovered many other substances which under the influence of animal tissues may be activated in the sense that hydrogen contained in their molecules becomes mobilized. Quastel in Hopkins' laboratory found that bacteria can supply the oxidase necessary to activate many different substances so that they would give up their hydrogen to oxygen. Hopkins believes that this mechanism may lend itself to the oxidation of lactic acid in muscle. His statement is that "a stimulus may bring about a transport of electrically charged enzyme particles and their concentration at the structural surfaces," in much the same way as Warburg conceives for the concentration of iron. This enzyme, moreover, instead of activating oxygen, activates hydrogen in the sense of making it available for union with oxygen in the recovery process after muscular contraction.

HYDROGEN CARRIERS

In certain kinds of oxidation hydrogen seems to be transported not directly from the foodstuff to be oxi-

dized but by intermediate steps. A substance capable of uniting with hydrogen may intervene as first an acceptor and later a donator of hydrogen so as to facilitate its passage from food to oxygen. One such substance has been isolated from yeast and from mammalian liver and muscle by Hopkins and has been given the name *glutathione* from the fact that it is composed of glutamic acid and cystein, which is a sulphur-containing amino acid. The sulphur in this substance may exist in one of two forms, S-H or S-S. The latter is called the disulphide form and the former the sulphydryl or thiol form. The disulphide readily takes up hydrogen from the oxidizable foodstuffs and transmits it to the oxygen supplied by the blood. It was thought at first by Hopkins that the S-H form was spontaneously oxidizable in the strict sense of the word, but it was later found by Harrison, working in Hopkins' laboratory, that traces of iron seemed to be necessary for the functioning of glutathione as a transporter of hydrogen.

It is quite possible that both of these theories are correct. That is to say, certain of the compounds liberated by the preliminary metabolism of our foodstuffs may be oxidized in the sense that oxygen is made ready for them, while certain others may be oxidized in the sense that they are made ready for union with oxygen. No final statement of the matter can be risked until much more information is gained concerning the chemical nature of the molecules actually burned in living tissues. Hopkins, who is probably the greatest living authority on the subject of tissue oxidation, believes that there is more evidence in favor of the activation of hydrogen than in favor of the activation of oxygen.

In closing this discussion a brief reference should be

made to Lavoisier, the great French chemist, who may more than any other man properly be called the discoverer of oxygen and its significance in nature. He it was who first identified oxidation in the body with ordinary combustion. With Seguin, his assistant, Lavoisier published in 1789 the first demonstration of the increased combustion caused by muscular work. He closes the memoir, entitled "Respiration in Animals," in the following words: "One sees that the animal machine is principally governed by three regulatory principles: respiration, which consumes hydrogen and carbon to furnish heat; transpiration, which augments or diminishes according as it is necessary to get rid of more or less heat; and digestion, which returns to the blood what it loses by respiration and transpiration."

Except that he believed the combustion took place in the lungs or blood instead of in the tissues, these views of Lavoisier would pass as very good physiology today. But note the spirit of the true scientist: "We will not conceal," he says, "one objection which may be made, indeed which we ourselves have made, against the theory here presented. No experiment tells us in a perfectly decisive manner that the carbonic acid gas which is disengaged during expiration is formed immediately either in the lung or in the circulation by the combination of oxygen of the air with carbon of the blood. It would be possible that a part at least of this carbonic acid should be formed in digestion; that it may be introduced into the circulation with the chyle; finally that, brought to the lung, it may be disengaged from the blood in measure as the oxygen is combined with it by a superior affinity. Experiments which we have already undertaken on digestion and transpiration

will probably clear up this doubt; they will dispel, we hope at least, the incertitudes which still remain on this subject. Perhaps then we shall be obliged to accept some changes in the doctrine which we have presented in this memoir. The modification of first ideas counts as nothing to those who search only for Truth for her own sake and without any other desire than that of finding her. We believe, however, we are not far removed from the time when, after having eliminated the remaining errors, the theory of respiration will not leave much more to be desired." One hundred and thirty-seven years have passed and physiologists and biochemists are still trying to eliminate the "remaining errors."

Chapter III—Article 2

THE NEED OF AIR: GOOD AND BAD AIR

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Dr. Phelps has held numerous appointments in sanitary science, including service in the Sanitary Research Laboratory of the Massachusetts Institute of Technology (1903-13) and in the U. S. Public Health Service as professor of chemistry (1913-19), preceding his present work in the College of Physicians and Surgeons of Columbia University. His special field is ventilation and he has served on important state, national and international commissions devoted to the study of this subject.

Air is the most intimate and least dispensable part of man's environment. It presses closely against all the external surfaces of the body and forces its way into the lungs, where it freely bathes a large internal surface. The circulation of the blood carries its component parts to every organ and tissue. It is not surprising, therefore, to find that health and comfort are dependent in considerable measure upon the atmospheric conditions. Let us briefly examine some of those conditions.

Chemically, air is a mixture of somewhat variable composition but containing approximately 21 per cent of oxygen, 78 per cent of nitrogen and 0.8 per cent of argon, by volume. Normally it contains also small and variable quantities of aqueous vapor and of carbon dioxide, together with still smaller traces of ammonia and the rarer gases, such as helium. The air of cities is often polluted with smoke and industrial fumes, while dust and bacteria, common impurities of most air, may

exist in harmful proportions under certain living or industrial conditions.

Physiologically, air has two primary functions. Its oxygen content is essential in metabolism in the same way that it is essential to combustion in a furnace fire, and its volume and movement are necessary to carry off the waste products of metabolism in the body, heat, carbon dioxide and aqueous vapor, just as the air of the chimney carries off the waste products of combustion in the furnace.

With these elementary facts in mind we may now, as a first approximation, define good air as air that is suited to the physiological requirements of the body, namely, the needs of metabolism and the disposal of the waste heat and gaseous products. It should be noted in particular that this definition applies to the air in immediate contact with the body and that air movement, by replenishing the near-by air supply, may change a bad air condition into a good air condition without changing the chemical composition of the air mass as a whole. Ventilation may similarly be defined as the art of maintaining the air of enclosed and occupied places in a condition suited to the physiological requirements of the occupants.

Without ventilation, or replacement of any kind, the air of occupied, enclosed places becomes vitiated and certain definite physiological symptoms result. For convenience we may refer to these results as the effects of vitiated air without going more fully into detailed descriptions or exact physiological definitions. The early symptoms, malaise, drowsiness, and flushing of the skin, are all too well known. Our present interest lies in the more exact determination of the specific causes of

these effects, in order that we may define good air conditions in more definite and more helpful terms.

CHEMICAL CHANGES

In their attempt to identify these causes, physiologists have quite naturally considered the various changes that have taken place in the air itself. In the first place breathed air is deficient in oxygen by about 4 per cent of the total air volume, so that repeated rebreathing of the same air would soon render it incapable of supporting life just as the air within a bell-jar becomes incapable of supporting the combustion of a lighted candle. The subject dies of asphyxiation. At the same time there is an equivalent increase in the content of carbon dioxide, a gas which, in sufficient concentration, is known to produce an unfavorable physiological reaction. It is natural, therefore, that vitiation of the air, as we ordinarily experience it, should have been regarded as merely a partial establishment of these extreme and fatal conditions. But it has been found by experiment that the effects of vitiation begin long before there is any serious reduction of the oxygen, and that men may live and work in perfect comfort in an atmosphere in which the proportion of the oxygen is much below that found in the most vitiated atmosphere of an overcrowded and unventilated room. The same facts are found in the case of carbon dioxide. Any waste product is naturally looked upon as unwholesome, if not actually toxic, but we find that the air of a room, otherwise satisfactory, may be contaminated with the carbon dioxide from an industrial process, to a much higher degree than is ever found under

conditions of poor ventilation, without doing appreciable harm.

A third possibility of chemical vitiation is not so readily disposed of. It has been thought, and with some apparent reason, that the exhaled breath may contain definite toxic substances, possibly organic in nature, and that the accumulation of these poisons, so minute in actual quantity as to elude chemical analysis or physiological test, may cause the symptoms that follow the breathing of vitiated air. Thus far there is no satisfactory evidence in favor of this view, although some evidence has been adduced. We may safely dismiss this possibility, however, as being at most a factor of minor importance in comparison with another phase of the matter concerning which we now have most satisfactory evidence.

IMPORTANCE OF PHYSICAL QUALITIES

In brief it is now known that the physical properties of the air are all important and that comfort and health under ordinary living and working conditions are determined primarily by the capacity of the atmospheric environment to cool the body rather than by its capacity to support combustion or to dispose of the gaseous products of exhalation. A single example of a series of classic experiments by the physiologist Flügge may be cited to illustrate this viewpoint. A human subject was shut up in an experimental chamber for four and three-quarter hours. During this time the temperature rose to 75° F., the humidity to 89 per cent of saturation and the carbon dioxide to 1.2 per cent. The subject suffered the usual effects of lack of ventilation, yet persons without the

chamber breathing the same air through respirators remained perfectly comfortable. The subject himself was not relieved by being permitted to breathe fresh air from without, while his body remained exposed to the conditions of the chamber, but was relieved when the temperature of the chamber was reduced by means of cooling coils, the chemical properties of the air, aside from its moisture content, remaining unchanged.

The present-day point of view has been admirably summed up by Professor Frederick S. Lee as follows:

"The evil effects exerted upon human beings by air that has become vitiated by human beings result not from a lack of oxygen, not from an increase in carbon dioxide, not from the presence of an organic poison, not from any chemical features of such air acting through the lungs on the tissues, not in any manner from the rebreathing of such air, but solely from the physical features of excessive heat and excessive humidity interfering with the proper action of the skin in regulating bodily temperature. The problem of bad air has thus ceased to be chemical and pulmonary and has become physical and cutaneous."

TEMPERATURE AND HUMIDITY

It appears, therefore, that in order to amplify further our provisional definition of good air it will be desirable to consider the physiological requirements of the human body, especially as regards the thermal relations. To this end it will be helpful to consider first some of the physical principles involved and then the physiological mechanisms of heat elimination from the body.

Within any solid mass heat tends to travel from a place of higher to one of lower temperature, just as water tends to travel down-hill. The analogy is quite complete. The rate of heat transfer depends upon the slope or gradient between the two places, which, in turn, is determined by the difference in heat level (temperature), and, inversely, by the distance between the two. Furthermore, just as the rate of flow of water is limited by the friction of the pipe or channel, so heat flow is retarded by the thermal resistance of the intervening medium. For convenience, however, we use the inverse of thermal resistance and characterize substances by their thermal conductivities. Metals are good conductors, having a low thermal resistance, while asbestos and air are examples of poor conductors.

The task of temperature regulation within the body may be expressed in physical terms about as follows. The body produces heat at an extremely variable rate. Taking as a convenient unit of reference the kilogram-calorie per hour per square meter of body surface, this rate varies from about 35 calories during sleep, through 50 calories at rest and up to 100 or more calories at hard work. In order to maintain a fairly constant internal temperature, these varying amounts of heat must be dissipated. A further variable is the temperature of the outer air, which even with the protection of clothing varies over a wide range. These two varying conditions are compensated by the temperature-regulating mechanisms of the body, located chiefly in the deep skin layers. The means of regulation are, first, a variable blood

supply to the surfaces, thus changing the effective thickness of the insulating skin, and second, a variable secretion of perspiration which, by its evaporation, cools the outer surface.

The cooling properties of the external air are determined both by its own temperature (ability to cool by conduction and convection of heat) and by its dryness (ability to cool by evaporation). Since the layers of air in immediate contact with the skin will quickly come to a condition of temperature equilibrium with the skin and also to a condition of saturation with aqueous vapor, heat loss from the body would cease in the absence of some air movement. A slight circulation of air about the body is always in effect owing to the tendency of the warmer and moister air to rise, but additional movement, such as that induced by fans, provides additional cooling power merely by bringing a greater volume of air into contact with the surface.

The three physical factors, therefore, temperature, humidity and movement of the air, determine its cooling properties. In a physical sense they are mutually interchangeable. That is, temperature may be increased and humidity decreased in such a way as to maintain a fixed cooling effect, and either of these factors may be modified and the effect of the change balanced by altering the velocity of movement.

The writer has made a determination of this physical equivalence in the cooling properties of airs of varying temperature, humidity and rate of movement. The three conditions were independently varied and the cooling power measured against a moist sheepskin surface.

A relation was developed which is best expressed by the following equation:

$$C=\sqrt[3]{V}\,[0.00185\ (37-t)+0.00802\ (43.44-h)]$$

in which

C = gram-calories of heat loss per minute per square centimeter of moist surface at 37° C.

V = velocity of air movement in centimeters per second

t = the air temperature (C.)

h = the air humidity in grams of aqueous vapor per liter.

Comparison of this formula, which expresses the physical facts of cooling from a moist warm surface, with the results of physiological tests shows that the physiological facts of cooling are of somewhat different nature. Thus, according to Atwater and Benedict, a man at moderate work may eliminate heat at the rate of 77 kilogram-calories per hour per square meter of body surface, expressed in the usual physiological units, or 0.13 gram-calories per minute per square centimeter, in the units of the formula above. Of this quantity it was found that about 69 per cent or 0.09 calorie, represented cooling by conduction and convection and the remainder, (0.04), cooling by evaporation.

The physical formula gives a cooling of 0.09 calorie from a surface at 37° C. exposed to air at 17.3° C., moving at 30 feet per minute (15 centimeters per second). But this same air if it were 50 per cent saturated with aqueous vapor would cool by unrestricted evaporation at a rate of 0.7 calorie as compared with the experimental value, 0.04.

Evidently evaporation from the human body is a controlled process and even under the assumed conditions of moderately heavy work its full physical cooling effect is not developed. Equivalent sets of physical conditions are not necessarily physiologically equivalent. This same conclusion may be derived from another line of reasoning.

Air at a temperature which induces copious perspiration, say 75° F., may be set in motion so that its actual cooling power, as measured by the total heat abstracted from the body, is the same as that of more quiet air at 68° F., but the feeling of comfort in the warmer air is not at all the same as under the cooler conditions. The cooling is by a different physiological process, one which puts more strain upon the regulating mechanism, so that despite the assumed equivalence in physiological cooling, in this case, there is still lacking a hygienic equivalence. An acceptable definition of good air, then, must contain certain terms other than those which merely define its cooling power, either in physical or in physiological terms.

INFLUENCE OF OCCUPATION

Nor is the physiological test of feeling equally warm a satisfactory criterion of equivalence in air conditions. In the first place the occupation of the individual has much to do with the sensation of equivalent cooling power. A person sitting at a desk in a cool air at low humidity would accept a considerable change in the humidity without experiencing any change in the sensation of warmth, because of the ready evaporation of the insensible perspiration regardless of the humidity change. In the same room, however, a person doing physical work and rely-

ing largely upon evaporation would be appreciably affected by a humidity change.

Again the clothing worn exerts a similar modifying effect, not only upon the actual rate of heat loss, but upon the sensation of equivalence when two air conditions are compared. Finally the matter of acclimation is of great importance. Indoor conditions which in the winter would be intolerably warm and unhealthful would be considered refreshingly cool in July.

For these and other reasons, many of which we perhaps have still to learn, good air conditions for occupied enclosed places can, for the present, be defined only in terms that have been worked out as a result of experience for the particular case in hand. Thus, for school rooms, the New York State Ventilation Commission has found, by extensive investigation, that a room temperature of not over 68° with normal winter humidity, which in the heated air of the class room is usually between 30 and 40 per cent of saturation, and with such a moderate interchange of air as can be had by the proper use of open windows, provides the optimum conditions for comfort and health.

The United States Bureau of Mines has, in a similar way, determined favorable and unfavorable conditions for miners at work in the air of mines which may be of necessity either very hot or very humid, and at times both hot and humid. Other investigators have studied other industrial conditions. It is only by such studies, based upon the comfort and general health of the subjects, industrial output, fatigue with its resulting accident rate, and upon similar physiological effects of an unfavorable environment, that a complete definition of good air may be obtained. Obviously such a definition

can never be freed from terms of occupation, clothing and perhaps other controlling factors.

For the home and office, however, and for any other situation in which the occupation and other conditions are comparable with those of the school, the standards of the New York State Commission are probably satisfactory, namely, not over 68°, without artificial humidification and with a moderate supply of fresh air from the open window.

For crowded places, theaters and auditoriums, definite provision must be made for the admission of fresh air by mechanical means. Even when this is done and an adequate supply of fresh air is furnished the good effect is too often undone by overheating. It is a matter of some surprise, to one who has not experimented with atmospheric conditions, how great an improvement in the conditions of a poorly ventilated office or a "stuffy" Pullman car may be obtained by merely dropping the temperature a few degrees. To a very large extent the so-called "poor ventilation" of living and working places is merely the result of overheating.

In brief, then, good air is primarily cool air, with a moderately low humidity and with sufficient movement and replenishment by fresh air from out-of-doors to provide for the normal cooling of the body.

CHAPTER IV

The Story of the Discovery of the Vitamins

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Dr. McCollum is a distinguished student of nutrition, who was a member of the faculty of the University of Wisconsin for ten years prior to becoming head of the Department of Chemical Hygiene at the Johns Hopkins University. He has made outstanding observations in relation to the science of nutrition, notably with reference to the vitamins, for which he originally proposed the modes of designation now in use. His book, "*The Newer Knowledge of Nutrition*," the latest edition of which was published in collaboration with Dr. Nina Simmonds, has enjoyed large popularity and is widely quoted as a statement of expert opinion on nutritional problems. He writes extensively on nutrition for the lay press. He is a member of the National Academy of Sciences.

IF ONE may judge from the popular discussions of foods and nutrition which are now enthusiastically read by the general public, the belief prevails that researches on vitamins are new. As a matter of fact philosophers in all ages have given thought to the nature of foods and the requirements of nutrition. The growth of knowledge in this field, like that in all other fields of science, was the result of attempts at experimental verification of philosophical speculation. The experimental period did not begin until about the beginning of the

nineteenth century. The period of philosophizing about foods began, so far as we know, with Hippocrates, the father of medicine. Even the conception of vitamins—substances which are required in but small amounts and until recently were too elusive for chemical study—dates back a century and a half, when James Lind, a surgeon in the British Navy, matured his studies on scurvy.

Hippocrates' (460-370 B. C.) view that there existed a specific universal nutrient principle (aliment) found in different kinds of natural foods probably represents the conception of how foods nourished the body with which thinking men had long been satisfied. Even as late as 1833, when William Beaumont, a surgeon in the U. S. Army, was engaged in his famous researches on the digestion of Alexis St. Martin, at an isolated military post in Wisconsin, the idea was still satisfying that there were many kinds of food but only one kind of "aliment." By this time Magendie (1783-1855), however, had begun feeding experiments on animals, using different kinds of foods. He restricted them to non-nitrogenous substances such as sugar, gum, olive oil, butter, etc. He exhibited great genius in his method of trying to find an answer to the question which is still stimulating scores of physiologists and chemists of today, viz., What, in a chemical sense, constitutes an adequate diet? To Magendie belongs the honor of establishing modern methods of research in nutrition. His differentiation between the value of nitrogenous as contrasted with non-nitrogenous foods is the outstanding landmark between the almost sterile two thousand years before and the fertile fields of modern physiology and biochemistry since his time.

Mulder (1802-1880) in 1839 clearly recognized the

similarity of albuminous substances of different kinds in animal and plant tissues; he coined the term protein to designate that class of nutrients which is now recognized as the principal structural element among the organic components of body tissues.

We can only mention here the achievements of Liebig (1803-1873) in advancing our knowledge of the chemistry of foodstuffs, and the great discoveries of Voit (1831-1908) in extending both the investigations of Lavoisier (1743-1794) on the energy needs of the body, and of Magendie on the necessity of protein. Near the end of the nineteenth century, Atwater (1844-1907) in his "Chemistry and Economy of Food," Bulletin 21, U. S. Department of Agriculture, 1895, formulated concisely the several phases of nutrition which seemed important to the most advanced thinkers of his time. These were: "the chemical composition of the materials used for the food of man; the proportions of nutritive ingredients; their digestibility; their fuel value; the ratios between their values for nutriment and their costs; the kinds of food and proportions of nutrients best adapted to the demands of people of different classes and occupations; the errors in our food economy; and the sociological and agricultural bearings of the subject." The most illuminating history of these phases of nutritional research is that of Dr. Graham Lusk, himself an eminent investigator of energy metabolism, in his book, "The Science of Nutrition." It will be noted that Atwater did not in 1895 include in his discussion the necessity of any of the principles now called vitamins, although studies long before published by Lind, Takaki, and Eijkman had shown their importance. Medical observers and pathologists appreciated

that certain diseases were related to diet long before they received attention from specialists in nutrition.

The limitations of chemical analysis when applied to such complex substances as plant and animal tissues, and the great complexity of these substances, made the problem of discovering what in chemical terms constitutes an adequate diet for a mammal one of extraordinary difficulty. It is one of the great triumphs of chemistry that workers in this field of science have been able within little more than a century to progress from a state of almost complete ignorance to our present creditable position. We are now able to explain the nature and cause of a number of specific kinds of malnutrition resulting from the restriction of either man or animals to certain kinds of food.

Until rather recently there has been a tendency to speak lightly of the pronouncements of scientists on matters relating to nutrition. In great part this was due to the fact that everyone has had personal experience with foods; each person generally has views based upon experience as to what proves for him to be a satisfactory diet. For many years investigators of nutrition problems have endeavored to explain human experience with diets of different kinds both for man and animals.

People in general, and even professional people with technical education, have been skeptical of the practical importance of the new discoveries about foods and nutrition. Many continue to believe that common experience has shown the safety of allowing the appetite, financial means, and custom to be the guide in the selection of food. The discoveries of the existence of hitherto unknown food principles, and the biological assay of all of our more common foodstuffs by appropriately

planned feeding experiments, have brought to light a number of specific kinds of malnutrition. These can be produced at will in animals; they are so strikingly like diseases which have long existed among human beings in certain places that they suggested the cause of the latter. The results of applying the knowledge of quality in foods to the study of human diseases has demonstrated that faulty diet has been and is the cause of much physical inferiority, illness and early mortality in infants and children; it has also been shown that careful attention to the food supply of the sick affords an effective means of cure in certain conditions and of relief and the extension of life in others.

The history of progress in nutritional research is a record of thought and experiment by many men for more than a century who without exception were inspired with a desire to learn the facts about the nutritive needs of the body and about quality in foods. The most noteworthy events in a chronicle of this branch of science will reveal how the vitamins were discovered and will show their importance in relation to health.

There had been in the literature of pathology for many years abundant evidence from human experience that a diet may have an appropriate chemical composition and yet be unsatisfactory for the nutrition of man. These observations did not secure from students of nutrition the attention they merited. It had been occasionally suggested for centuries that certain diets of stale foods produced outbreaks of scurvy among sailors, soldiers and prisoners. It was also known that fresh vegetables and fruits would promptly cure scurvy.

HISTORY OF EARLY PROGRESS

Scurvy.—Scurvy is a disease in which the outstanding feature is the destruction of the capillary vessels with resulting hemorrhage. There are few recorded data concerning the existence of the disease previous to about 1550. So far as we know the first recorded account of scurvy is found in the history of the crusade of Louis IX in the thirteenth century. The French army suffered greatly from it at this time. Many of the sailors with Vasco da Gama in his voyage off the coast of Africa in 1498 were greatly afflicted by scurvy. Hirsch in his "Handbook of Geographical and Historical Pathology," 1885, records 144 outstanding epidemics of scurvy between 1556 and 1877. In general it attacked armies, sailors, prisoners, and the inhabitants of besieged cities. The civilian population of Russia has regularly suffered from scurvy during the long winter season.

Lind records in his "Treatise on Scurvy," 1757, a careful study of all the available information relating to the disease. He was convinced that the disease was due to a lack of fresh vegetable food in the dietary. Notwithstanding the irrefutable nature of his evidence the question of the etiology of scurvy continued to be debated almost to the present time. The kind of soil, the influence of wet and cold, the restriction of man to a diet containing an excess of acid radicals, and the possible infectious nature of the disease, were the principal causes suggested.

So long as knowledge of scurvy rested entirely upon human experience there were definite limitations to the demonstration of the relation of the diet to the disease. Exposure to cold and wet, excessive fatigue, a diet of

cooked or dried foods, and the frequent occurrence of epidemics under conditions which were likely to bring about an epidemic of scurvy, tended to produce confusion in the minds of people as to its etiology. Physicians and lay observers frequently confused malaria and other diseases with scurvy. A few capable observers and careful students, however, had long ago become convinced that diet is the alpha and omega of the etiology of scurvy. There is no old literature relating to scurvy in infants. It appears that the disease did not afflict infants until the development of the pasteurization of milk and the beginning of bottle feeding.

Beriberi.—The etiology of a deficiency disease which afflicted the rice eaters of the Orient for many centuries remained a puzzle until about 1880. The earliest description of beriberi dates from about 2600 B. C., but it was known to the Chinese before that time. The most notable among the early investigations relating to the etiology of beriberi is the work of Takaki, Director-General of the Japanese Navy. Between 1878 and 1883 the average number of cases treated medically in the navy was 323.5 per thousand per year. The principal components of the diet of the sailors was polished rice and fish, the remainder being derived from a number of vegetables. Takaki made a study of the diet and health of the British sailors and decided that beriberi was due to faulty diet. He endeavored to change the diet of the Japanese sailors. This was accomplished in 1884. The improved dietary resulted in practically a disappearance of the disease among the sailors.

The etiology of beriberi was discovered by Eijkman between 1889 and 1897. He observed that chickens developed the essential features of beriberi when restricted

to polished rice as their sole food. Grijns, Hulshoff-Pol, and Vordermann deserve much credit for their contributions which led to the full establishment of our knowledge of the etiology of this very serious human disease.

In 1923 the Franklin Institute of Philadelphia presented a medal to Dr. C. Eijkman, Emeritus Professor of Hygiene at the University of Utrecht, in recognition of his great services to humanity in the discovery of the first experimental procedure for producing in animals a "deficiency disease" due to lack of a vitamin in the diet. Professor Eijkman was not able to come to America, so the Dutch Minister from the Netherlands, the Hon. Jonkheer A. C. D. de Graeff, accepted the medal in his behalf. On this occasion he related his own observations in 1897, which showed in a spectacular manner the result of Eijkman's studies on birds of the effect of restricting them to a diet of polished rice. A part of his address on that occasion is quoted. "I witnessed personally the complete change which Professor Eijkman's researches and experiments had brought about in the sanitary conditions of our colonies in the East Indies. As a young colonial official I lived at Buitenzorg in the neighborhood of the so-called beriberi hospital, and daily I saw hundreds of the poor sufferers of that mysterious disease passing my home in batches. It was a pitiful sight. Natives, Chinamen and a few white men dragged themselves along with their swollen legs. They had to take a daily walk, as exercise in the fresh air, if possible, was in those days the panacea for this disease, wherefore no other cure was found. Many of the numerous cases in the crowded hospital were not even able to walk; they died slowly within the pre-

cincts of the hospital, and more than once it happened that some of the patients having their daily exercise collapsed and died on the road of heart failure.

"So was the situation in 1895, and some years later the hospital at Buitenzorg was closed for want of inmates, and beriberi had become an illness with almost only historical importance. It was Dr. Eijkman who as a humble medical officer accomplished this wonder by his earnest researches."

Although beriberi is still sufficiently important from the public health standpoint to make necessary periodic international congresses, and to be the subject of much discussion by officials of several oriental countries, there is no question about the possibility of prevention of the disease by proper diet. The only difficulties in the way of eradicating it lie in the lethargy of unprogressive and unenlightened peoples.

FURTHER CLUES AS TO THE NEED OF VITAMINS IN OUR DIET

In 1881 there appeared a paper by Lunin in a standard scientific journal in Germany which should have aroused the deepest interest of every physiologist and biochemist working on nutrition problems. Lunin compared the growth of mice fed on milk with those fed a diet composed of milk sugar, milk fat, milk protein, and the mineral constituents of milk. He found that although adult mice could live in apparently good health for several months on the milk diet, they invariably died within one month on the synthetic mixture. He drew the conclusion that "other substances indispensable for nutrition must be present in milk besides caseinogen, fat, lactose, and salts." Although Bunge

discussed these experiments in his widely used textbook, "Physiological and Pathological Chemistry," they did not attract the attention they deserved.

Following Lunin's suggestive work, Socin in 1891 conducted experiments of a similar nature; ten years later several other names were added to the list of those who endeavored to nourish animals on diets composed of isolated and more or less purified proteins, carbohydrates, fats and salt mixtures. They likewise found that mammals could not be kept alive on such diets.

The early researches on scurvy and beriberi were essentially medical rather than chemical or physiological in their viewpoint. From the early work of Liebig, who gave the first effective impulse to the systematic investigation of the chemistry of foodstuffs, chemists and physiologists have steadily added increments of knowledge year by year to the chemistry of the carbohydrates, fats, and proteins, and their physiological rôles in the body. These have been spoken of as the three great staminal principles essential in nutrition. The German agricultural chemists perfected a system of food analysis which was generally believed for about forty years to yield results of importance, viz., the relative values of different natural food products. The more advanced thinkers realized, of course, that their knowledge concerning dietary properties of foods and nutritional needs of the body was of a provisional nature and would ultimately be modified and extended by further researches. It is worthy of note, however, that these specialists in nutrition do not appear to have given attention to the well-demonstrated relation of diet to the diseases, scurvy and beriberi.

The indifference of close students of nutrition to any

information which was not yielded by the chemical analysis of the time is shown by the finality with which Atwater, the outstanding investigator in America in this field up to 1900, recommended to housewives certain practices relative to the purchase of foods. In Bulletin 21, U. S. Department of Agriculture, 1895, he contrasted the nutritive values of a list of common foods in terms of amounts of proteins, fats, carbohydrates and energy which could be purchased in the Eastern states for twenty-five cents. He stated: "The figures of the table tell their story so plainly that they need very little comment. A quarter of a dollar invested in the sirloin of beef at 22 cents per pound pays for one and one-seventh pounds of the meat with three-eighths of a pound of actual nutritive material. This would contain one-sixth of a pound of protein and one-fifth of a pound of fat, and supply 1,120 calories of energy. The same amount of money paid for oysters at the rate of 50 cents per quart brings 2 ounces of actual nutrients, an ounce of protein, and 230 calories of energy. But in buying wheat flour at $5.00 a barrel the 25 cents pays for 6.25 pounds of nutrients with eight-tenths of a pound of protein, and 11,755 calories of energy. The price of food is not regulated solely by its value for nutriment. Its agreeableness to the palate or to the buyer's fancy makes a large factor of the current demand and market price." It will be seen from this and other statements in this bulletin that he was inclined to speak lightly of the various adjuvants to the daily diet, and to regard their purchase as mere indulgence in pleasure.

Atwater and Bryant in 1906 tabulated in Bulletin 28 of the U. S. Department of Agriculture the existing analytical data relating to the composition of American

foodstuffs for the guidance of all who were interested in evaluating foods, without commenting in any way on the inadequacy of a diet containing only those nutrients listed in their tables. Atwater's views represent the manner in which the subject of nutrition was taught previous to 1911. He did not express any doubt as to the physiological adequacy of even the simplest or most monotonous dietary provided it supplied the approved amount of protein, carbohydrates, fats and calories which chemical analysis determined. He made no mention of the hazards of taking a diet restricted to a few foods, nor of the danger of inducing what are now known as deficiency diseases by a simple and monotonous food supply.

Pekelharing, of the University of Utrecht, expressed in 1905 the belief that an adequate diet must contain substances which were still unknown, since he found that mice when fed a bread composed of casein, albumin, rice flour, lard, and a mixture of salts, and were given water to drink, starved to death. One will recognize that these are essentially purified food substances, free from any of the vitamins.

Phosphorus.—One of the possible causes for failure of nutrition of animals restricted to a diet of isolated and to some extent purified food substances such as Lunin and others had studied was believed by several investigators (among them Hall, 1896; Steinitz, 1898; Leipsiger, 1900; Zadik, 1899; and Ehrlich, 1900) to be found in the inability of the body to assimilate and utilize for all purposes inorganic forms of phosphorus. This idea was probably suggested by the fact that the principal proteins of milk and of egg yolk contain phosphoric acid as a part of their molecules. These sub-

stances are prepared in the body for the specific purpose of the rôle they are to play in the early subsistence of the young. For fifteen years prior to 1912 efforts were made to establish experimentally whether lecithins, phosphorized proteins and the other organic compounds of phosphorus which occur in the body could be synthesized from inorganic phosphorus compounds. The proof that these could be synthesized was demonstrated between 1909 and 1912.

Up to 1909 the most important problem which experimenters had met in the feeding of rations composed of purified food substances was loss of appetite in their animals. McCollum introduced a new feature into this kind of study by seeking to increase the variety of foodstuffs in the diet. He hoped in this way to overcome the failure of others to secure satisfactory nutrition in rats. In the series of experiments referred to the one condition required of every organic component of the ration was that it should be free from phosphorus in any form. This condition was practically met; the only source of phosphorus in the diet was finely ground tricalcium phosphate, a natural mineral. Several young rats gained in weight on this diet, one somewhat more than doubling its weight in fifty-six days. The only difference between the experimental diets used in this study and those of the earlier investigators appeared to be in the palatability. Some years later it became apparent that the cause of the success of this experiment lay in the chance inclusion in the diet of milk sugar which contained what is now known as vitamin B, and butter fat which contained at least traces of all the fat-soluble vitamins. The details of this study have been described elsewhere and will not be repeated here.

Vitamins in Fats.—In 1909 a most important discovery was made by Stepp, who demonstrated that diets from which all of the lipoids or fat-like substances had been extracted were no longer capable of supporting the life of adult mice. The introduction of any of the known constituents of fats, such as cholesterol, lecithin, etc., did not restore the diet to completeness. He was unable to identify the nature of the indispensable substance which fat extraction of his diets had removed. Restoration of the alcoholic extract of certain foods such as egg yolk did make the diets complete for mice. We now know that his alcoholic extracts contained all the fat-soluble vitamins and also some vitamin B.

Some Notable Feeding Experiments.—An experiment which stimulated much interest in nutritional research and established the fact that very marked differences existed in the nutritive value of two or more rations which yielded closely similar results on chemical analysis, and which possessed almost the same digestibility, was carried out at the University of Wisconsin between 1906 and 1911. The object was to determine whether rations for cattle so made up as to be alike in so far as could be determined by chemical analysis, but derived each from a single plant, would prove to be of equal nutritive value for growth and the maintenance of vigor.

The ration employed for one group of animals was derived solely from the wheat plant, and consisted of wheat straw, wheat gluten and the entire wheat grain. The ration of a second group consisted of the entire corn plant, which included the kernel, stalk, and the leaf, together with a portion of corn gluten, a by-product of the cornstarch industry. The third group

obtained their ration solely from the oat plant, being fed entirely on rolled oats and oat straw and leaf. There was a fourth group, which it was supposed would serve as controls, that was fed a ration having the same chemical composition, but derived from about equal portions of wheat, corn and oat products.

The animals employed were young heifer calves weighing about 350 pounds, and were as nearly comparable in size and vigor as could be secured. They were restricted absolutely to the experimental ration. They were given all the salt they cared to eat, were well cared for, and were allowed to exercise in an open lot free from vegetation. Their behavior during growth and during the performance of the functions of reproduction was extremely interesting. All groups ate practically the same amounts of feed, and digestion tests showed that there were no significant differences in the digestibility of the three rations.

It was not until the animals had been confined to the experimental rations for a year or more that differentiation in their appearance was easily observable. The corn-fed group was sleek and fine, and evidently in an excellent state of nutrition. In marked contrast stood the wheat-fed group. These animals were rough-coated and gaunt in appearance, and small of girth as compared with those fed the corn-plant ration. The weights of the two groups did not differ in a significant degree. The groups fed the oat-plant ration and the mixture of the three plants, leaf and seed, stood intermediate between the lots just described. The assumption that the animals receiving the mixture of products would fare better than the others, and thus serve as the standard group for controls, did not prove correct. The corn-fed animals were

at all times in a better state of nutrition than were those receiving the greater variety of food materials.

The reproduction records of these animals are of special interest. The corn-fed heifers invariably carried their young to full term. The young showed remarkable vigor, and were normal in size and able to stand and suck within an hour after birth, as is the rule with vigorous calves. All lived and developed in a normal manner. The young of the wheat-fed mothers were the reverse in all respects. They were born three to four weeks too soon, and were small, weighing on an average 46 pounds, whereas the young of the corn-fed animals weighed 73 to 75 pounds each. The latter weight is normal for new-born calves. The young of the wheat-fed mothers were either dead when born or died within a few hours. The young of the mothers which had grown up on the oat-plant ration were almost as large as those from the corn-fed mothers, the average weight being 71 pounds. All of the cows in this group produced their young about two weeks too soon. One of the four was born dead, two were very weak and died within a day or two after birth. The fourth was weak but with care it was kept alive. The young of the cows which were fed the mixture of the three plant products were weak in most cases. One was born dead and one lived but six days. The mothers were continued on the experimental rations and the following year they repeated in all essential details the reproduction records observed in the first gestation periods.

Records were kept of the milk production during the first thirty days of the first lactation period. The average production per day per individual of the corn-fed lot was 24.03 pounds; for the wheat-fed animals, 8.04; and

for the oat-fed animals, 19.38 pounds. Those fed the mixture of the three plants produced an average of 19.82 pounds of milk per cow per day during the first thirty days. In the second lactation period the average figures for milk production were 28.0, 16.1, 30.1, and 21.3 pounds, respectively, per day during the first thirty days.

Through autopsy and analysis of the tissues of the young, and analysis of the feeds and excreta of the animals of the several groups, an elaborate attempt was made to solve the problem of the cause of the marked differentiation of the animals fed these restricted diets. Interesting data were secured which showed marked differences in the character of the fat in the milk of the cows of the different lots. The observation was made that the urine of the wheat-fed animals was invariably distinctly acid in reaction, whereas that of the other lots was alkaline or neutral. It was not possible by any means known to biological chemistry to work out a reason as to the cause of the pronounced differences in the physiological well-being of the different lots of cows.

During the years 1911-1915 Osborne and Mendel reported many experiments with diets consisting of purified food substances except for the inclusion of about 28 per cent of "protein-free milk." This material was prepared by removing as completely as possible the casein, albumin, and fat from milk, and evaporating the resulting whey to dryness. This was found to be a most important adjuvant to the mixture consisting otherwise of purified foodstuffs. This diet, especially when butter fat was included, proved to be essentially complete for the promotion of growth and the maintenance of health in their rats. Using this diet they were able to make significant studies of the relative biological values of

many proteins. When, however, they substituted a mixture of inorganic salts and purified lactose for the "natural protein-free milk" they secured less satisfactory results and some of their rats developed an ophthalmia, which they found cleared up when the animals were given butter or cod liver oil.

During the years Osborne and Mendel studied the nutritive values of different proteins, McCollum and Davis continued to study the cause of failure of young rats which were fed diets of purified foodstuffs. It was assumed by some investigators that the diet with which McCollum had secured growth in 1909, and which consisted of edestin of hemp seed, zein of maize as its proteins, corn and wheat starch, *milk sugar*, glucose, cane sugar, butter fat, bacon fat and cholesterol, together with a mineral supply, the only source of phosphorus being tricalcium phosphate, was simply a mixture of proteins, carbohydrates, fats and mineral salts. When attempts were made to repeat this experiment *in principle*, with the use of purified proteins, starch, cane sugar, agar-agar, a salt mixture, and lard, the result was prompt failure of the animals. No one was then able to see the significance of the difference in the composition of the two food mixtures. McCollum and Davis undertook to determine wherein the difference lay.

Differences in Fats.—In 1913 McCollum and Davis reported their demonstration that with a certain diet growth could be secured when butter fat or egg yolk fats were included, but the animals failed when lard or olive oil was the only fat supplied. Soon after, Osborne and Mendel reported similar results; they secured growth with butter fat or cod liver oil, but not with lard or almond oil. These studies showed clearly that

some fats contain one or more nutrient principles not contained in certain other common food fats. Up to this time it had been generally accepted that all fats were merely sources of energy and were essentially equivalent in their nutritive values irrespective of their source.

Closing in on the Vitamins.—It happened that the experimental diets with which this demonstration of the remarkable difference in the nutritive values of fats was made contained what we now call vitamin B. In the diet of Osborne and Mendel this was furnished by the "protein-free milk" and in that of McCollum and Davis by milk sugar. The milk sugar was believed to be essentially pure, and so for a time McCollum and Davis believed that the only chemically uncharacterized nutrient in their diet was that in the "growth-promoting" fats. Funk had by this time (1911) discovered the old experimental work of Eijkman (1897); he had coined the term *vitamine* to designate the anti-neuritic principle which is removed with the polishings in the preparation of polished rice. Belief in the need for such a substance as Funk's vitamin in the nutrition of the rat was difficult to maintain in the light of the demonstrated fact that young rats had been made to grow well on a diet of isolated foodstuffs which were regarded as essentially purified, except for the presence of the unknown principle in certain fats which in these experiments determined whether success or failure would be the outcome. During the years 1911-1914, however, evidence had accumulated from various laboratories which suggested the necessity of two unidentified nutrient principles in the diet, but up to 1915 no clear-cut demonstration could be made that this view was correct. The final proof came through the efforts of McCollum and Davis

to discover the cause of nutritive failure of animals fed upon single grains (whole wheat and polished rice). They found that wheat is rendered complete for the nutrition of the rat by supplementing it with three things, viz., a salt mixture, a protein to enhance its amino-acid content, and a fat which furnished the "growth-promoting" principle mentioned above (now called vitamin A). Polished rice, however, did not become complete from the nutritive standpoint by the addition of these three known food principles.

It will be recalled that in 1909 McCollum had secured growth in rats fed a diet (page 124) containing butter fat, but which appeared to consist otherwise of foodstuffs which were pure, and which supplied only proteins, carbohydrates and mineral salts. They were, therefore, much mystified by their failure to secure growth in rats fed polished rice supplemented in the same manner as had rendered whole wheat complete. Rice, they believed, could only be a mixture of protein, starch, and mineral salts, and according to their theory of what constituted a satisfactory diet, should have been made complete from the nutritional standpoint by the addition of a purified protein, a mineral salt mixture, and a fat containing the properties of butter fat or cod liver oil. They were therefore forced to return to the study of the diet of purified foodstuffs. They could easily see that the only vulnerable point in its composition was the kind of carbohydrate which it contained. The successful diet contained milk sugar, whereas the unsuccessful ones which appeared to be entirely comparable in composition contained starch. They prepared a diet containing purified protein, starch, milk sugar, butter fat, and mineral salts in appropriate

amounts, and compared its effects on animals with one composed of the same materials except that the milk sugar was replaced by dextrinized starch. The former had some growth-promoting power, the latter none. It then appeared that something in the milk sugar was of importance, and it remained to be determined whether the sugar itself or some impurity in the commercially pure product made the difference in the experiments described. It was found that when the milk sugar was specially purified it was only the equivalent of starch in its dietary properties, whereas milk sugar not specially purified proved far superior in its dietary value. The mother liquors obtained from recrystallizing impure milk sugar had a marked beneficial effect in inducing growth when added to the diet of purified food substances containing a growth-promoting fat.

Fat-Soluble Vitamin A and Water-Soluble Vitamin B.—From these results it was evident that there were necessary in the diet two hitherto unidentified substances, one of which is soluble in fats and insoluble in water, the other insoluble in fats and soluble in water. McCollum and Davis formulated in 1915 a working hypothesis as to what in chemical terms constituted an adequate diet. They stated that the diet must contain in addition to the long recognized dietary factors, viz., protein, a source of energy in the form of proteins, carbohydrates, and fats, a suitable supply of certain inorganic salts, and two as yet unidentified substances or groups of substances. One of these, vitamin A, is associated with certain fats and is especially abundant in butter fat, egg yolk fats, cod liver oil, and the fats of the glandular organs such as liver and kidney, but is absent or present in but traces in fats or oils of vegetable

origin. The second substance, vitamin B, is never associated with fats or oils of either animal or vegetable origin. It is widely distributed in natural foods.

Other Vitamins.—This hypothesis contained all that could be said at that time about the needs of the rat for dietary essentials hitherto unappreciated. As further work progressed it became apparent that other vitamins existed. The application of the method for the biological analysis of a food, formulated by McCollum and Davis from their studies of wheat and rice, has led to the classification of foodstuffs on the basis of their dietary properties. It was soon demonstrated that the leaf of the plant has dietary properties very different from and superior to those of the seed, tuber, root or fruit.

An understanding of the properties of individual foods as to their value in terms of specific nutrient principles makes possible the combination of several foods, each deficient in one or more respects so that what is lacking in one will be supplied by another. Much study has been given to the supplementary values of combinations of two or more foods. Attention has been focused upon the inorganic requirements of the animal body; the study of the kinds of malnutrition which result from feeding faulty combinations of foods has also attracted much experimental inquiry. The most striking results of this many-sided investigation, however, are the specific types of pictures of disease which result from a deficiency of one or another of the vitamins in the diet. A detailed account of vitamin research would be beyond the scope of this paper. We shall conclude with a brief recital of the outstanding advances relating to this class of food principles.

OUR PRESENT KNOWLEDGE

Vitamin A Deficiency and Injury to Epithelial Tissues.—Several observers (including Falta and Noeggerath, 1906; Knapp, 1909; Freise, et al., 1913; Osborne and Mendel, 1913) noticed in poorly nourished rats the occurrence of a conjunctivitis with crust formation. Osborne and Mendel referred to the condition as a "type of nutritive deficiency exemplified in the form of an infectious eye disease prevalent in animals inappropriately fed." They found the condition to be speedily cured by the introduction of butter into the diet. Mori (1904) described 1400 cases of xerophthalmia among children in Japan which he believed to be due to faulty nutrition. He called attention to the household remedy of chicken livers or eel fat in the treatment of the condition.

During several years McCollum and Simmonds (1917) collected numerous observations on the eye disease in question. All of these were indicative that the disease was non-infectious and that it could be more satisfactorily explained on the basis of specific starvation for vitamin A than on any other. We correlated these observations on rats with those reported by Bloch and by Mori, and came to the conclusion that the xerophthalmia or keratomalacia produced experimentally in animals is the analogue of the condition which these authors had reported in man. We formulated the view that this type of xerophthalmia is a manifestation of a deficiency due specifically to lack of vitamin A.

Mori (1922) studied histologically the eyes of rats in various stages of specific starvation for this factor. He found the first observable change to be a tendency

for the lachrymal glands to go into a resting stage and to cease to produce tears. When the supply of tears fails the conjunctival sac is no longer washed continuously as under normal conditions, and bacteria quickly begin to grow there in great numbers. Leucocytes soon accumulate in the eye chamber, causing the well known hypophyon which is visible as a yellowness in the pupil. Through their dissolution albuminous material accumulates, forming a sticky exudate which tends to paste the eyelids together. This drying of the exudate makes it difficult for the animals to open their eyelids. The Meibomian and Harderian glands are also affected.

Wolbach and Howe (1925) made a study of the changes of the epithelial tissues throughout the body. They found that xerophthalmia results in the transformation of various epithelia into a stratified, squamous, keratinizing epithelium. This change is practically constant in the upper respiratory tract, including the whole of the nasal passages, larynx, trachea and bronchia. All the salivary glands are affected and frequently show cavities resulting from the retention of desquamated, keratinized epithelial cells. They found no changes in the stomach and intestine. In the genito-urinary tract this transformation into keratinized epithelium is found in the renal pelvis, bladder, seminal vesicles, epididymus and prostate gland. In all the glands noted there was a considerable degree of atrophy before a change in character of the epithelium took place.

Distribution of Vitamin A.—Vitamin A is found most abundantly in cod liver oil, butter fat, egg yolk fat, liver, leafy vegetables and yellow pigmented vegetables generally. The most curious fact about its dis-

tribution is its absence in vegetable structures which do not contain yellow pigments.

The vitamin is readily oxidized at higher temperatures, but is relatively stable under the conditions generally maintained in the cooking of foods.

Recent Advances in the Study of Vitamin B.—As was pointed out in connection with the early studies on beriberi, it is now generally accepted that the disease is in some way the result of adherence to a faulty diet; one which consists largely of polished rice is especially likely to produce it. The symptoms due to peripheral neuritis are relieved by the provision of some substance which is extracted by either water or dilute alcohol from many natural foods, especially yeast, wheat germ, rice polishings or the leaves of several plants. Some close students of beriberi are unwilling to admit that the disease is due entirely to lack of a substance which for years has been called vitamin B; they insist that some other factor or factors operate together with vitamin deficiency in its etiology. This view gains support from the fact that beriberi occurs in two distinct forms, the wet and the dry, and that these may occur in the same individual at different times in the course of the disease. It is well known that no case of beriberi has ever developed when the food supply is satisfactory.

Efforts to Obtain the Pure Principles.—For many years efforts have been made by several investigators to separate the preparations containing vitamin B (antineuritic substance) into two or more substances. Certain results obtained in the cure of polyneuritis in pigeons and in inducing growth of young rats on diets of purified food substances containing fats which supply the fat-soluble vitamins have suggested that the anti-

neuritic potency of certain foods does not parallel their growth-promoting power when these serve as the sole source of vitamin B. A careful study of the experimental data bearing on this subject leaves one with the conviction that the data are somewhat suggestive, but in no instance do the results demonstrate the multiple nature of the preparations which show high potency in both kinds of experiments. Extremely potent preparations of the vitamin (?) have been prepared by a number of workers (Grijns, Susuki, Funk, Seidell, Levene, Jansen, McCollum and Simmonds, Osborne and Wakeman, and others), but with perhaps the single exception of Jansen there is no convincing evidence that but a single chemical substance is responsible for the physiological properties which they exhibit. Jansen has described a preparation from rice polishings which he has tested in the form of several crystalline derivatives. Each exhibits the appearance of a pure chemical compound which is very potent in relieving the symptoms of polyneuritis in birds. He believes that a single substance, the antineuritic principle, when included in a diet composed otherwise of purified food substances and fat-soluble vitamins, is capable of maintaining health in birds. He has not tested his preparations for promoting growth in rats.

A most interesting suggestion, based upon experimental evidence, has been made recently by Goldberger and his associates (1926). They believe that pellagra is a vitamin deficiency disease and that the preventive substance is a component of the fraction hitherto designated as vitamin B. They state that the antineuritic principle (active in preventing beriberi) is destroyed by heat at fifteen pounds pressure (about 118° C.) in

three hours, whereas the pellagra-preventive principle is stable under these conditions; that the pellagra-preventive principle is much less soluble in cold 85 per cent alcohol than is the antineuritic substance. It is left behind in great measure when the antineuritic substance is extracted from maize meal by percolation with 85 per cent alcohol. In experiments with rats they found that neither the alcoholic extract alone nor large amounts of yeast which has been autoclaved were capable of inducing growth or maintaining life in rats, whereas much smaller amounts of the two combined stimulate growth and maintain health. Several investigators have already repeated or modified their experiments and accept their interpretation. On the other hand, Eijkman has examined the purified crystals prepared by Jansen and asserts his belief that their potency fully establishes the individual nature of the principle. Only through further investigations can it be established beyond doubt whether the so-called vitamin B fraction contains one or more than one indispensable principle.

Vitamin C and Scurvy.—Modern knowledge of the etiology of scurvy begins with the classic experiments of Holst and Fröhlich, who in 1912 demonstrated that guinea pigs readily develop scurvy when restricted to a diet of cereals or bread. They pointed out that the picture of disease thus produced is the analogue of scurvy in man. It is characterized by loosening of the teeth, inflammation of the gums, hemorrhages into the joints, decalcification of the epiphyses of the long bones, and the characteristic changes in the bone marrow. The addition of small amounts of fresh cabbage, carrot, or other fresh vegetables induced recovery from the disease.

They also observed that the exclusive feeding of guinea pigs with carrot, turnip, or dandelion did not lead to this condition, although the animals lost comparable amounts in weights (34-40 per cent) for want of sufficient food. They explained their observations on the theory that scurvy results from the absence in the diet of an antiscorbutic principle which is destroyed in most foods by the ordinary processes of cooking.

The Importance of Raw Food.—As a result of the experimental work on animals by Holst and Fröhlich and by many investigators since their results were published, we have a most valuable body of knowledge concerning the importance of raw food in the diet. It is not essential that a very large part of the food supply should consist of uncooked vegetable food, but a certain amount should be regularly included for the purpose of supplying the antiscorbutic principle now called vitamin C. Lind stated that total deprivation of men for about forty days of all fresh fruits or vegetables would result in the appearance of incipient scurvy. Nothing is definitely known concerning the minimum amount of vitamin C which will just suffice to maintain optimal health, so the best practice would seem to be to eat daily at least a small amount of one or more articles known to possess antiscorbutic value.

The Diet in Relation to Rickets: Vitamin D.—Although rickets in infants and children was described by Glisson in 1650, and believed by him to result from unsatisfactory hygienic conditions, the cause of the disease was little understood until it became possible in 1921 to produce it experimentally in animals. It has been demonstrated that an unsatisfactory relation among three dietary factors—calcium, phosphorus and an or-

ganic substance called vitamin D—may produce the typical pathological changes in the bones of growing animals. When vigorous young rats with a good physical inheritance are used the disease can only be produced in the absence of sufficient vitamin D, together with unfavorable relations between phosphorus and calcium in the food. When vitamin D is lacking in the diet rickets develops when the phosphorus of the food is very low and the calcium disproportionately high. It also develops when the conditions are reversed, viz., when the calcium is very low and the phosphorus in the food is disproportionately high. When these relations between calcium and phosphorus are maintained but suitable amounts of vitamin D are supplied, e. g., as in the form of cod liver oil, the typical bone lesions of rickets do not appear although the bones may not be normal. Under certain conditions osteoporosis may result.

The Importance of Sunlight.—Early observations on the geographical distribution of rickets showed that the disease is rare in the Tropics and in the Arctic regions; it was essentially restricted to the North Temperate Zone. It gradually became known that mountain sunlight was capable of preventing the disease in children and of initiating the healing of the lesions of rachitic children. Experimentally it has been demonstrated that young rats may be kept on a rickets-producing diet without developing the disease if they are exposed to sunlight, or are irradiated with the rays of an ultraviolet lamp. Even irradiation of the food mixture prevents the development of rickets. This remarkable observation has received careful study by a number of investigators. It is now asserted by eminent authorities

that cholesterol of ordinary purity is the only component of the diet which can be "activated" by ultra-violet light. When highly purified it can no longer acquire the property of preventing the disease. The view now prevails that a sterol of fungi, especially from ergot, called ergosterol, is the principle which is activated by light so as to acquire the properties of vitamin D.

Since it has been shown experimentally both on infants and rats that rickets is a disease resulting from a lowering of the phosphorus, and to a lesser extent of the calcium of the blood, which interferes with the calcification of the growing bones, there is much interest in the question of what constitutes the optimal content of the diet in these two elements. The work of the last few years has shown that no absolute values can be recommended, since when there is an abundance of vitamin D present,* or when the skin is irradiated with light containing a suitable content of ultra-violet rays, normal calcium and phosphorus metabolism will be maintained, even when the proportions of the bone-forming elements are unfavorable. In the absence of the influence of these agencies it becomes of great importance that the content of the diet in calcium and phosphorus should be properly adjusted.

Vitamin E and Fertility.—In 1922 Evans and Bishop published a remarkable series of experiments. They found that on certain diets rats could grow to full maturity and appear normal, but were incapable of reproduction. They demonstrated that a certain type of sterility is a dietary deficiency disease, since it can be cured or prevented by a change in the dietary

* Unpublished data.

regimen. This change involves the addition of certain single natural foods high in a new food factor, vitamin E, or of the addition of much smaller amounts of extracts of these foods. The sterility disease affects males and females differently. In the male they found it leads to destruction of the germ cells, and eventually the entire seminiferous epithelium. This is not the case with the female, where the ovary and ovulation are unimpaired throughout the sexual life; a highly characteristic disturbance occurs in gestation, namely, the death and resorption of the developing young.

They point out that in gestation where vitamin E is low or absent, the embryos seem at first normal but often by the eighth day a retardation in development can be demonstrated. Evident abnormality, especially monstrosity, does not occur. At some time between the twelfth and twentieth day fetal death occurs, usually on the twelfth or thirteenth day, but for some days after, the maternal part of the placenta continues to live. There may also be continued gain in the mother's weight until the twentieth or twenty-first day. This, they state, would appear to speak decisively for a peculiar need on the part of the developing young for the new vitamin as against placental injury as the cause of death. It would appear that the maternal placenta is not altered structurally sufficiently to justify the conclusion that its function has been impaired. Mason (1926) has made a detailed study of the histological changes in the testes resulting from deficiency of vitamin E. This vitamin is found most abundantly in the lipoid extracts of cereal grains, but is abundant also in various kinds of leafy vegetables.

VITAMIN DEFICIENCY AND RESISTANCE TO INFECTION

The experience of investigators in nutrition has been that their experimental animals on vitamin-deficient diets are far more likely to suffer from infections than are normally fed animals. The studies relating to specific phases of immunity as influenced by deficiency of one kind or another in the diet have been conflicting. It is still open to question as to how far the observed lowered resistance to infefction is the result of general nutritional impairment.

The latest branch of medicine to develop is that of preventive medicine. This new field owes much to animal experimentation. Nutritional research has a greater value in this phase of medicine in raising the vitality of mankind with all that this implies than it has in the prevention of the occurrence of the deficiency diseases. In the aggregate by far the most important effects of faulty nutrition in man are the result of errors of one kind or another which are not sufficiently grave to cause prompt and spectacular failure. It is the gradual operation of more or less constant but unperceived causes rather than great exposures of an accidental nature which in most cases is responsible for undermining the health of the individual. Of these causes it now seems certain that the consumption of an improperly constituted diet is one of the most important. It is one of the causes of inferiority in physical development, instability of the nervous system, lack of recuperative power and endurance, and consequent cumulative fatigue, and lack of resistance to infections such as tuberculosis and other types where specific immunity is not easily developed by the body. Natural vitality

enables the tissues to resist the invasion of certain bacteria; an optimal condition of the tissues constitutes an effective defense for the body against certain kinds of diseases.

There is a tendency, because of the prominence given to vitamins in all popular literature relating to nutrition, for many to gain the idea that the prevention of a deficiency of these principles is regarded by those who are closest to research in this field as the problem of primary practical importance. This is far from the truth. The adequate diet is fairly complex chemically. All who are fully conversant with the vast body of experimental studies which have accumulated during the last three decades fully appreciate the significance of considering the diet as a whole, with its thirty-five or more nutrient principles, and the short-sightedness of elevating the vitamins to an exalted position at the expense of other equally important dietary factors in any discussion of the subject of human or animal nutrition.*

* The authors have discussed the practical aspects of planning the daily dietary in accordance with the results of modern research in a popular book, "Food Nutrition and Health." Published by the authors, Box 25, East End Post Station, Baltimore, Md.

CHAPTER V

The Conquest of Dietary Diseases

Article 1

NO CHILD NEED HAVE RICKETS

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Preceding his present service in the Harvard Medical School (1922), Dr. Gamble was associated at the Johns Hopkins Medical School (1915-22) with the late Dr. John Howland, an outstanding leader in the study of rickets with the aid of chemistry. Dr. Gamble is an authority on the metabolism of inorganic substances (salts) and the chemical changes which are the basis of rickets.

THE Structure of Bone.—The essential feature of the disease known as rickets is a defect in the development of bone. Because of its simple structural purpose bone would seem to be the least interesting of body tissues. Bones, however, have long been admired by students of body mechanics for their extraordinary accuracy of design. Even the finer texture of the varied units of the framework which supports the weight of the body and sustains the pull of muscles is wrought with a wonderful perfection in terms of its purpose. In Plate I is shown a photograph of a section of the upper part of the thigh bone. At first glance we are not likely to discover anything architecturally remarkable. We know that bones are hollow and perhaps we know that a

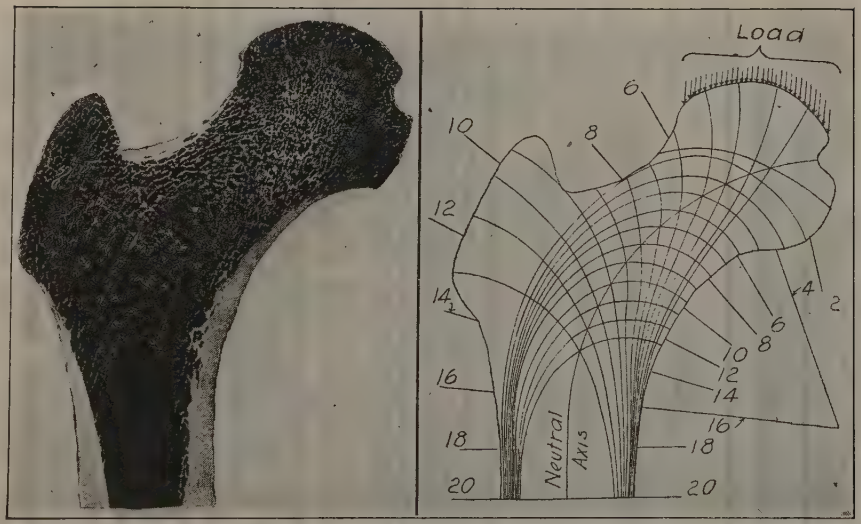


PLATE I. THE ARCHITECTURAL ACCURACY OF THE STRUCTURE OF BONE. PHOTOGRAPH OF LONGITUDINAL SECTION OF HEAD AND NECK OF FEMUR, AND DIAGRAM SHOWING LINES OF STRESS AS CALCULATED MATHEMATICALLY. (KOCH, IN THE *AMERICAN JOURNAL OF ANATOMY*.)

hollow cylinder is, for the same amount of material, many times stronger than a solid one. We have noticed too the meshwork appearance of bone below its outer shell. Adjacent to the photograph is a diagram of the upper end of the femur in which have been drawn the lines of stress to which this bone is subjected by the weight of the body. These lines were calculated mathematically and, as may be seen by reference to the photograph of the femur, they describe accurately its chief structural features; the hollow shaft and the "spongy" bone of the head, the increasing thickness of the wall of the shaft and the relatively thin shell of the head. Moreover it may be noted in the diagram that the calculated lines of stress tend to cross each other at right angles. In the photograph we find that the spaces of the delicate meshwork formed by the intersecting trabeculæ of bone are approximately rectangular. We thus learn that the arrangement of the stone used in the construction of bone is, to the smallest detail, mathematically correct.

The Growth of Bone.—The architectural perfection which an adult bone presents is the result of a long and intricate process of development, the phases of which, as studied microscopically, constitute a fascinating chapter of histology. We may here only briefly and inadequately indicate the steps in the process of the growth of bone. In the case of the long bones this takes place chiefly near the ends of the shaft. Here we find traversing the bone of a thin zone of richly cellular tissue known as osteoid tissue and on either side of this a region in which the cells are more and more widely separated by a stiff gelatinous intercellular substance. These cells are "old" osteoid cells, marooned and nearly lifeless in a sea of

extra-cellular substance which they have secreted. This transformed osteoid tissue is described as cartilage and it is in the ground substance of cartilage that the stonework of bone is begun. Here lime salts are deposited. But this is simply a first laying in of material like the lumber pile beside a house under construction. The calcified cartilage is subsequently invaded by minute blood vessels from the richly vascular central cavity of bone. These vessels excavate the cartilage, absorb and redistribute calcium salts, with the ultimate result that we find in adult bone calcified trabeculæ arranged both as regards direction and density to coincide, with beautiful accuracy, with the lines of stress which the particular bone must sustain.

The increase in length of a growing bone is thus the result of a rapid multiplication of cells in the zone of osteoid tissue. Transformation of osteoid into cartilage keeps close pace with cell proliferation so that the region of growth remains a mere line across the bone near either end. Owing to the almost microscopic thinness of this "growth line" and the almost immediate calcification of the cartilage continuously produced from it, growing bones are sufficiently rigid to serve their structural purposes. If, however, the deposition of lime salts does not closely follow the formation of cartilage, more or less extensive regions of insufficient rigidity develop near the ends of the bones. This is the situation described as rickets. The thrust of body weight and the pull of muscles on these abnormally flexible parts of the bones produce the bodily deformities which so conspicuously indicate the presence of this disease. A familiar example is "bow legs" (see Plate II). A very characteristic evidence of rickets is a series of lumps at the junctions of

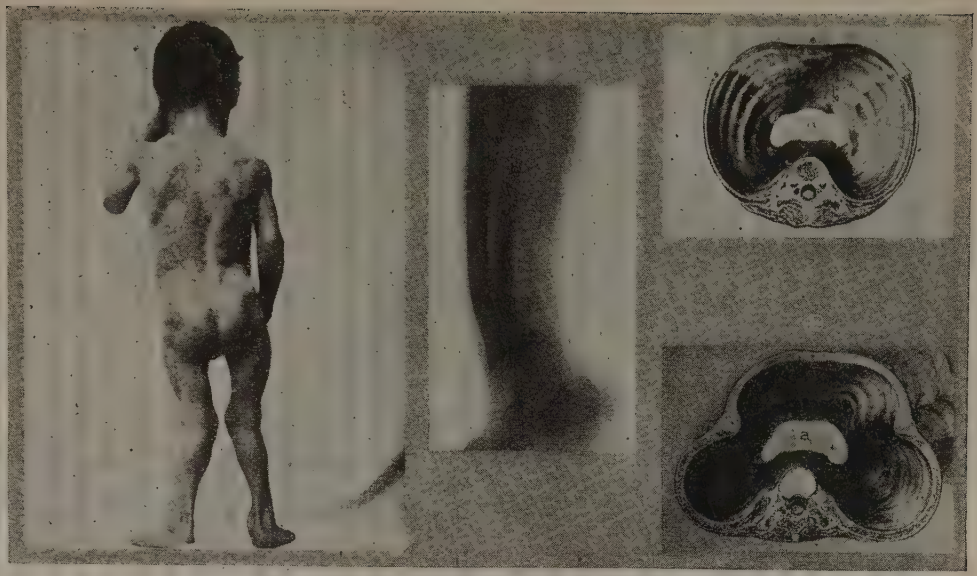


PLATE II. DEFORMITIES OF THE BODY FRAMEWORK DUE TO RICKETS. THE UPPER OF THE TWO DRAWINGS OF THE CROSS SECTIONS OF THE CHEST SHOWS THE NORMAL SHAPE OF THE CHEST CAVITY; THE LOWER DRAWING IS THE SHAPE IN RICKETS.

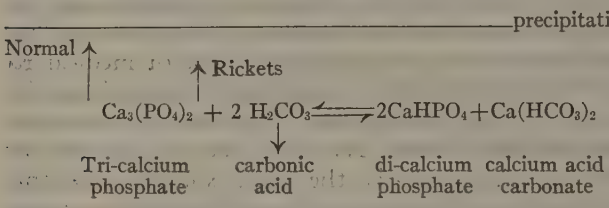
the ribs with the breast bone forming the "rachitic rosary" (see B on Plate II). Change in the usual contour of the cranial bones producing a "square" forehead is often prominent. There is usually quite marked thickening near the ends of the long bones. There may be serious change in the shape and capacity of the chest cavity and marked bowing of the spine. These various results of an incorrect construction of the framework of the body are shown in Plate II. Rickets being the result of the failure of calcification of cartilage to keep pace with the rate at which it is produced, we may expect the disease to occur with greatest frequency during the period of most rapid growth, and we find in fact that rickets rarely develops after the first two years of life.

The ultimate goal of the study of a disease is to be able to describe its cause and explain the resulting disturbance of physiological processes in terms of chemistry. If so complete a knowledge can be obtained we may assume almost with certainty that directly curative measures can be devised. As yet no disease has been analyzed to this point of theoretical thoroughness. In several diseases (diabetes is a notable example), however, the chief factors are now so well in hand in terms of chemical processes as to permit an almost accurate control. Rarely do we find the basal chemical defect so easily visible as in rickets. By simply looking through a microscope we learn at once that rickets is due to failure of a normal process of chemical change, viz., the precipitation of lime salts in the cartilage of growing bone. The stonework of bone is almost entirely composed of a single chemical substance, tri-calcium phosphate. The problem of rickets was long ago defined by

Lehnerdt's question: *"Warum bleibt das rachitische knockengewebe unverkalkt?"* (Why does the rachitic bone tissue remain uncalcified?) Explanation of abnormality in a physiological process must obviously be based on an understanding of its mechanism under normal circumstances. So that here we must undertake to answer an antecedent question: What are the factors which normally bring about a precipitation of calcium salts in the cartilage which the osteoid tissue produces?

The Chemistry of Bone Formation.—Thus openly does rickets invite study in terms of chemistry, and the results so far obtained are excellently illustrative of the method and of the rewards of this type of medical research. We have then first to explain a normal physiological event, the precipitation of tri-calcium phosphate in the cartilage of bone. As initial data we know that tri-calcium phosphate is the least soluble substance which the body fluids contain and that in the blood plasma which carries it to the bones it is present at a concentration not far below the saturation level. We also have evidence that the composition of interstitial fluid throughout the body, that is, fluid lying between tissue cells, is closely similar to blood plasma, the fluid which floats the blood corpuscles. Evidently, however, some factor, or factors, in the matrix of cartilage differ from their values in the blood plasma in a direction which produces precipitation of tri-calcium phosphate. The evidence of *in vitro* experiments suggests that the precipitation of this salt may be due to a lower concentration of carbonic acid in the cartilage than obtains in the blood plasma. If a solution of all of the inorganic substances which the blood plasma contains be prepared, the concentration of each being accurately as in

normal plasma, it will be found that if the carbonic acid factor is then appreciably lowered, a precipitation of tri-calcium phosphate at once takes place. There are certain reasonable grounds for supposing that the concentration of carbonic acid in the matrix of cartilage is actually lower than in blood plasma. The mechanism of the normal precipitation of tri-calcium phosphate in the bones, according to this theory, which was suggested by Howland and Kramer, may be briefly described with the help of the following equation:



Here on one side of the equation is tri-calcium phosphate and carbonic acid and on the opposite side two other calcium-containing salts, di-calcium phosphate and calcium acid carbonate, which the blood plasma also carries and which we may note are much more soluble than tri-calcium phosphate. The equation represents a balanced situation. A change in the size of a factor will induce compensating changes in the values of the other factors in such manner as to restore balance between the two sides of the equation. Upon arrival of these substances in the cartilage matrix there occurs reduction of the carbonic acid factor. This produces a disturbance of balance which tends to be restored by increase of the companion factor tri-calcium phosphate at the expense of di-calcium phosphate and calcium acid carbonate.

The increase of tri-calcium phosphate brings it to its saturation level so that to such further extent as it is formed it must precipitate.

The Failure of Bone-Formation in Rickets.—If we have here correctly explained the precipitation of tri-calcium phosphate under normal circumstances how may we imagine that this process fails in rickets? Looking at the equation we see two possibilities. Perhaps the reduction of carbonic acid does not take place in rickets. We have, however, no evidence in support of such a surmise. A much more likely possibility would be a less than usual amount of the materials, di-calcium phosphate and calcium acid carbonate, from which the increase of tri-calcium phosphate is constructed; and this we know to be actually the case in rachitic blood plasma. The theory just described has recently been substantiated by most ingenious *in vitro* experiments in which calcification of slices of rachitic cartilage from animals was obtained according to its terms.

The failure of precipitation of tri-calcium phosphate in rickets is, then, referred to a lack in the blood plasma of the materials from which this substance can be constructed. This finding was anticipated for a long time before it was established. The construction of tri-calcium phosphate requires two building units, calcium ion $Ca++$ and phosphate ion $(PO_4)^{\equiv}$. Effort was first centered in the attempt to demonstrate that in rickets the blood plasma carries less than the usual amount of calcium. For a number of years the results of a great many studies were not in agreement owing to unsatisfactory methods for measuring calcium in the very tiny samples of blood plasma which must be used. Finally, however, as "micro" methods of analysis became suf-

ficiently accurate, the fact was established that there is really little or no lowering of plasma calcium in rickets. Calcium in blood plasma is normally remarkably stationary at 10 to 11 milligrams per 100 cc. In rickets it is sometimes as low but rarely lower than 9 milligrams. This was a great disappointment. "Failure of calcification" so strongly suggested lack of calcium. Joy returned, however, when the other equally essential component of tri-calcium phosphate, viz., phosphate ion $(PO_4)^{\equiv}$ was thought of. Only a few years ago Howland and Kramer in this country and, independently, Iversen and Lenstrup in Denmark discovered that the concentration of phosphate ion in the blood plasma is greatly lowered in rickets. This factor, measured as phosphorus, is normally 5 to 6 milligrams per 100 cc. In rickets it is often found below 2 milligrams.

Initial Factors Leading to Rickets: the Rôle of Vitamin D.—We have then, as the proximal cause of the failure of calcification of the bones in rickets, an insufficient amount of phosphate ion in the blood plasma. What are the initial factors in the pathogenesis of the disease which are responsible for this lack of bone-building material in the blood stream? During the past ten years, owing to the discovery almost simultaneously by several groups of workers in this country and in England that rickets can be produced in animals by certain diets, this question has been extensively and industriously studied. There has been a brisk competition in the attempt to identify in chemical terms the dietary factors responsible for experimental rickets. To state briefly the results of these studies, *two* dietary defects are necessary in order to produce rickets in young animals. There must be an abnormality of the proportions

of calcium and phosphorus in the food and this must be accompanied by an almost complete lack of an as yet unidentified organic substance which has been designated as vitamin D. As regards the calcium-phosphorus intake it has been found that an excessive amount of calcium and a very small amount of phosphorus is the most dependable adjustment of these factors for the production of experimental rickets. The effect of a large amount of calcium in the food probably is to block completely absorption from the digestive tract of such phosphorus as the food contains by combining with it to form the difficultly soluble tri-calcium phosphate. The factor, vitamin D, although not yet in hand as a substance of known chemical constitution, has been shown to be contained only in certain natural foodstuffs such as animal fats, butter and the yolk of eggs. This substance is abundantly present in cod liver oil. If young rats are placed on a diet of high calcium and low phosphorus content and lacking in D, but which in all other respects is a complete and correctly balanced food for them, after an interval of several weeks there will be found in the blood plasma of these animals a large reduction of phosphorus, and if the bones be examined microscopically the newly formed cartilage will be found empty of tri-calcium phosphate. In other words the picture of human rickets is completely reproduced. If, after young rats have been rendered rachitic in this manner, a small amount of cod liver oil be placed in the diet a most remarkable event takes place. In spite of there being practically no phosphorus in the diet the concentration of phosphate in the blood plasma rises within a few days to its usual value and if the bones are then examined the evidences of healing rickets are

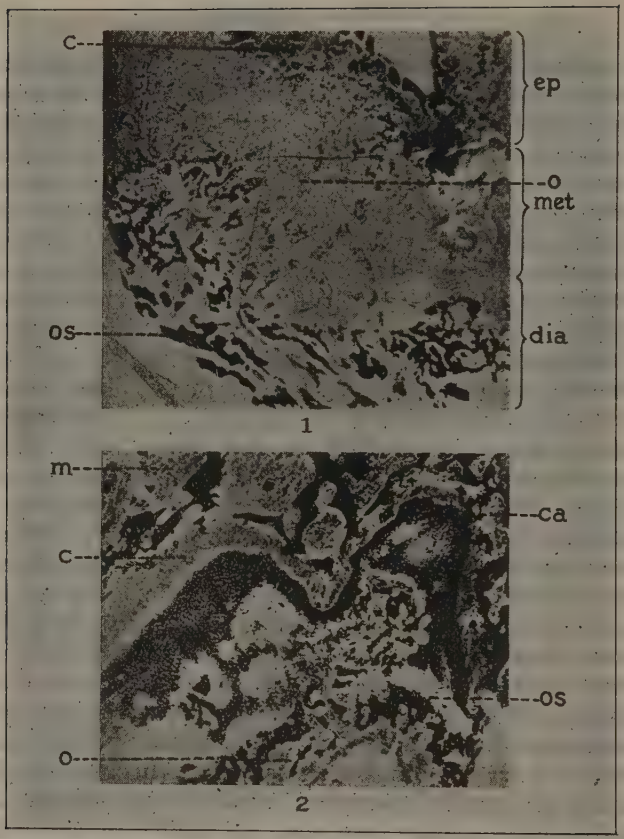


PLATE III. THE DIRECT EVIDENCE OF THE RICKETS CURATIVE ACTION OF COD LIVER OIL. PHOTOMICROGRAPHS OF SECTIONS OF CARTILAGE FROM RATS. THE UPPER SHOWING ACTIVE RICKETS AND THE LOWER HEALING RICKETS FOLLOWING THE ADMINISTRATION OF COD LIVER OIL. THE INTENSELY BLACK AREAS IN THE LOWER PICTURE INDICATE CALCIFICATION. (SHIPLEY, PARK, McCOLLUM, SIMMONDS AND PARSONS IN THE *Journal of Biological Chemistry.*

found. The histological proof of the rickets curative action of cod liver oil is strikingly shown by the two micro-photographs of sections of bone tissue from rats in Plate III. In these pictures the intensely black areas indicate calcification. In the upper one, which portrays active rickets, may be seen a wide space quite empty of lime salt deposits. The calcified trabeculæ surrounding this region were in existence before the experimental diet was begun. The lower picture shows the effect of adding cod liver oil to a diet which has produced rickets. The extensive precipitation of tri-calcium phosphate which a few days of cod liver oil administration has produced is described by the densely black dotted region. This is the microscopic picture of healing rickets.

The results of experimental rickets emphasize the fact that the outstanding feature of the disease is an incorrect metabolism of phosphorus rather than of calcium. Bones and calcium, however, seem to remain obstinately associated in the medical as well as in the lay mind, with the result that organic calcium salts are still extensively prescribed with the hope of protecting the bones and teeth. As we have seen above an excess of calcium in the food tends actually to prevent calcification by causing a precipitation of tri-calcium phosphate in the gastro-intestinal tract instead of in the cartilage of bone. The charm of euphony is so great, however, that the slogan "Calcium for calcification" will long be heard.

Rickets and Sunlight.—Besides the identification of the two dietary defects concerned in the causation of rickets, recent intensive study of the disease has produced the important discovery that lack of an environmental factor, sunlight, is also of direct pathogenic sig-

nificance. It has been thoroughly shown that the rays producing the ultra-violet portion of the solar spectrum exert a rickets curative effect which is apparently identical with that of the food substance D. For instance, if rats rendered rachitic by diet are placed in sunlight for several hours daily there occurs exactly the same restoration of lowered blood plasma phosphorus and the same histological picture of healing rickets as would be produced by adding cod liver oil to the diet. So that we have for contemplation the spectacular fact that a correct adjustment of phosphorus metabolism produced by ingestion of the food substance D may also be obtained by permitting sunlight to reach the skin of the body. The inference that the substance D exerts a photochemical form of activity is substantiated by the recent important experiments of Hess and of Steenbock which demonstrate that various vegetable oils and certain other foodstuffs ordinarily therapeutically inert can be rendered rickets curative by irradiation. Rickets research has thus brought us to the threshold of the study of the rôle of photo-chemical reactions in physiological processes.

Human Rickets Prevented by Vitamin D and Sunlight.—According, then, to the results of these recent studies of rickets in animals the requirements for the production of the disease are an unbalance of the calcium-phosphorus intake together with a lack both of the food substance D and of sunlight. How do these causative factors for experimental rickets agree with the circumstances under which human rickets occurs? We may not immediately assume that the production of the disease in animals by a given set of factors explains the pathogenesis of human rickets. Apparently the abnor-

mality of the calcium-phosphorus intake, which may be described as the predisposing factor, is of relatively less significance in human rickets. Recent studies of the composition of the food given infants and also of the dietaries of nursing mothers in the stratum of our larger communities where rickets is prevalent have shown that the amounts and proportions of calcium and phosphorus are sometimes incorrect but to an extent very much less than is necessary for the production of rickets in animals. We may of course make here the easy inference that our species is more susceptible to rickets than the race of rats. We can, however, provide a much more commendable explanation by recalling that rats cannot be placed on the type of experimental diet used for producing rickets until after they have been weaned at an age of several weeks. They have then nearly passed their period of "infancy" and correspond, as regards stage of growth, to children two years or more of age. Now, after the first two years of life, children become rapidly insusceptible to the development of rickets. It is therefore probable that a much larger discrepancy as regards the calcium-phosphorus intake is necessary for the production of rickets in rats past the age of infancy than would be required during the initial period of much more rapid growth. Indeed it is not unlikely that the predisposing factor is during infancy relatively insignificant and that lack of D and sunlight may produce rickets even when the calcium-phosphorus intake is practically correct.

As regards these two deficiencies, lack of D and of sunlight, we have excellent evidence that they may be dependably regarded as the essential factors in the causation of human rickets. The geographical distribution

of rickets clearly indicates the significance of sunlight. In latitudes sufficiently south so that infants and young children may wear a minimum of clothing and be out-of-doors the year round, rickets is practically unknown. As we go northward in the Temperate Zone, rickets becomes rapidly more prevalent, and here we find further striking evidence indicating the sunlight factor in the sharply seasonal incidence of the disease, its development being confined almost exclusively to the winter and spring months of the year. These are the relatively dark months, and also during this period, because of cold weather, infants are often kept nearly continuously indoors. A recent study of rickets in India by Hutchinson and Shah is clearly illustrative of the preventive rôle of sunlight. Surely there should be no rickets in India. Yet the infants of the top-caste Hindus almost always develop extremely severe rickets. Owing to the religious custom of "purdah," which compels their mothers to live beyond the public gaze, these infants are practically never taken out of dusky rooms. In contrast the infants of Hindu laborers who cannot afford so expensive a religious detail practically never develop rickets. The women work with the men in the fields and leave their infants alongside in the sunlight. The rickets preventive action of sunlight is here particularly clear cut, since in both instances the dietaries of the mothers almost entirely lack the foodstuffs which contain the substance D. That D if abundantly present in the food will prevent rickets even in the complete absence of sunlight is demonstrated for us by the rickets-free infants of the Esquimaux.

In temperate climates, where rickets is after all most common, we may probably correctly assume that the

food of infants, which is usually either mother's milk or cow's milk, does not as a rule contain quite enough of the substance D to protect alone against rickets. If, however, infants are not continuously screened by non-transmitting window glass from the curative rays in sunlight, rickets does not develop. Relatively brief periods out-of-doors permit these rays sufficiently to supplement the protecting action of D in the food. Rickets is thus seen to be the result of man's ingenuity in providing protection by means of clothing and houses against harsh changes in external physical circumstances. These inventions permit his existence in periodically severely cold regions. In the coziness of his artificial environment he has felt no direct consciousness of biological sin, although in recent times he has occasionally heard preachment of the bodily morality of "fresh air" and "out-of-doors." Rickets should definitely convert him.

In times of prosperity with adequate supply of usual foodstuffs we may regard rickets as practically always due to an insufficiency of exposure to sunlight during the winter months. During wars or periods of severe economic distress from other cause, extensive dietary deficiency of D explains the greatly increased prevalence of rickets, the foodstuffs which contain D being unfortunately very much the most costly. Rickets is therefore a disease of poverty as well as of darkness. Foote has most interestingly called attention to the great frequency with which the deformities of rickets are clearly depicted in the paintings of the German and Flemish primitives. The social circumstances in the towns of northern Europe during medieval times—dark dwellings, recurring wars with resultant periods of poverty and semi-famine—must unquestionably have sustained an exten-

sive prevalence of rickets. Were the infants of that period so regularly rachitic that artists were unconscious of deformities in their models? Such an inference is not an improbable one. Even in our better times, in the poorer sections of urban communities, a large part of the infant population is rachitic. No other disease is nearly so prevalent during the first two years of life.

Concomitant Defects of Rickets.—It may be noted here that the significance of rickets as a disease is by no means completely described by the conspicuous deformities of the bony framework of the body which are its outstanding feature. These do not endanger the life of the infant. Only in severe cases is there a residuum of permanent distortion and mechanical disability. Occasionally this costs the life of a mother at childbirth. But in the great majority of instances the marks of the disease are scarcely discernible in after years. Rickets presents, however, besides the conspicuous defect in bone growth, other features which indicate a general disturbance of nutritional processes. There is marked anemia, flabby musculation and impairment of digestive function. Rachitic children are notoriously predisposed to dangerous gastro-intestinal disturbances and to the contraction of infectious diseases, especially of the respiratory tract. And since rickets is so widely prevalent it is undoubtedly indirectly responsible for a large part of infant mortality.

Rickets Practically Annihilated.—Recent research, then, informs us that lack of sunlight and of a certain food substance are the chief causative factors in this "largest" disease of infancy. Of the manner in which these agencies bring about a correct control of the concentrations of calcium, and especially of phosphorus, in

the body fluids we have as yet no knowledge. The data so far obtained simply demonstrate this control. We may, however, confidently believe that the invention of experimental rickets provides a method of study which will finally produce an understanding of rickets in full chemical detail. But, as has often been the case, the bravery of the attempt to resolve a disease into its ultimate chemical terms has been most graciously rewarded. In advance of a complete conquest of detail, the salient defenses of the disease have been taken. With a knowledge of its chief causative factors in hand, rickets is practically annihilated.

This splendid significance of the results of recent studies of rickets is not in the least subtracted from by admitting that the knowledge thus gained is not new knowledge. That cod liver oil will cure rickets has been known for several hundreds of years. This discovery is credited to fisher folk of northern Europe. That sunlight is preventative was convincingly evident to students of rickets generations ago. In recent times, however, the introduction into medicine of a commendably cautious attitude of reliance only on directly demonstrable data has produced a perhaps too inclusive disrespect for the older empirically established beliefs. A splendid clearance of rubbish has been accomplished but, inevitably, grains of gold have been lost with the dross. To an even mildly critical attitude of mind the belief that oil from the liver of codfish is specifically curative of a human ailment was obviously a quaint bit of archaic medical lore. The theory of a relationship of sunlight to prevention seemed an interesting but intangible surmise. The scientific method of thinking having produced skepticism, it was quite necessary that the

rickets-preventive action of cod liver oil and of sunlight be rediscovered in irreproachably scientific terms. That this has been most beautifully done ensures a permanent and active belief in the efficacy of these agencies. And since their employment is so easily practicable we may now regard rickets as a preventable and unnecessary disease. Already the incidence of the disease has been extensively reduced. Faith in the applicability of methods of chemistry to the discovery of the factors of disease has thus conquered a serious scourge with which man has for thousands of years unconsciously afflicted his offspring.

Chapter V—Article 2

THE DISAPPEARANCE OF SCURVY

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Clinical Professor of Pediatrics, University and Bellevue Hospital Medical School, New York

Dr. Hess has been Clinical Professor of Diseases of Children at the University and Bellevue Hospital Medical College since 1915. His book, "*Scurvy, Past and Present*," is an authoritative exposition of the subject. He is widely recognized as one of the foremost investigators of rickets and is well known for his discoveries relating to the influence of sunlight and other forms of radiant energy on the development of the bones and on other features of physiologic well-being. Latterly he has demonstrated that many foods and specifically ergosterol, a distinct chemical compound, can be rendered antirachitic by exposure to ultra-violet rays.

Scurvy was known to Hippocrates, who refers to the large number of men in the army who suffered from pains in the legs and gangrene of the gums, symptoms accompanied by prostration and loss of teeth. The Greek, Roman and Arabian writers, however, do not seem to have been acquainted with it. In the thirteenth century we find an account of scurvy by de Joinville, who accompanied the Crusaders in their invasion of Egypt under St. Louis. He describes the havoc which this disorder played among the soldiers and gives a graphic description of their debility and the lividity and spongy conditions of the gums and their tendency to bleed. In the seventeenth century things had come to such a pass that the Faculty of Medicine at Copenhagen published a "consilium" for the benefit of the poor, on the causes, prevention and cure of the disorder, which was prevalent among the Danes and other northern

nations. A century later the colonists of the northern part of America were sorely afflicted with scurvy. It is stated that the French suffered such high mortality during the severe winters in Canada that they frequently debated the wisdom of abandoning the settlement. It is, however, in war that scurvy has always claimed its greatest toll; for example, in the Russian armies, in the war between the Austrians and Turks in 1720, among the English troops who had taken Quebec from the French in 1759, and among the French soldiers in the army of the Alps in the spring of 1795. In regard to the War of the Rebellion Munson writes: "Scurvy prevailed among our troops during the Civil War and its recognition was a surprise and shock to professional ideas preconceived from practice in civil life." The same was true in the Russo-Japanese War of our time. In the recent World War reports published after hostilities had ceased showed that many thousands of troops along the eastern front had been incapacitated by scurvy, and that it also played an important rôle among the soldiers on the western front. Nor was the civilian population of the various warring countries spared, more particularly in Russia and in Austria.

It is doubtful, however, whether attention would have been focused so sharply on scurvy had it not been for the voyages of exploration undertaken in the sixteenth century. These long trips on sailing vessels, where for many months little or no fresh vegetable or animal food was obtainable, seemed almost designed to make a test of the dietetic origin of scurvy. The great feat of Captain Cook, who sailed the seas for three years without having a sailor come down with scurvy—he fed liberal rations of "sweetwort" and sauerkraut—was

a demonstration which could not be disregarded by the British marine. But the lesson was slowly learned. Although the English naval hygienist, Lind, extolled the value of lemons and oranges, of sour cabbage and of all fresh fruits and vegetables, the naval hospitals continued to be filled with sailors suffering from scurvy. The merchant marine of England was still more conservative, and for many years after scurvy had been eradicated from the navy, we still read of its occurrence on the vessels making voyages to India, China and Ceylon.

The Importance of Fresh Food.—Scurvy is dependent on the amount of fresh food which is consumed by people, and is therefore especially rife during the winter time. It is a danger even today. In most civilized countries the potato stands as the bulwark which prevents the occurrence of scurvy, and outbreaks frequently follow a failure of this crop. It is important for us to realize that we are still dependent on annual crops for our protection from scurvy, that we are leading a hand-to-mouth existence in regard to our supply of essential antiscorbutic foods. Ireland and Norway have been particularly the victims of such a hazardous condition. About ten years ago the eastern part of the United States suffered a mild outbreak of scurvy following a partial failure of the potato crop.

Scurvy and Babies.—During the past twenty-five years the main interest in this disorder has shifted to infantile scurvy, frequently termed "Barlow's Disease." This is a disorder which was described in the middle of the seventeenth century by the illustrious Glisson: but his account passed unnoticed. It occurs in bottle-fed babies who receive for many months a diet limited to heated cow's milk, with or without cereal addition. The

babies grow pale and fretful, fail to gain in weight, evince tenderness of the extremities and perhaps hemorrhages of the gums. The greatest danger, however, lies in the fact that a marked susceptibility to infections is associated with this nutritional disturbance. About twenty-five years ago this deficiency disease of infants made its appearance in France due to the fact that industrialized milk—*"lait maternisé"* and *"lait fixé"*—came into vogue. The former of these preparations is subjected to various manipulations and finally heated to a temperature above the boiling point; the latter is shaken violently in a machine to render the fat globules smaller and then is sterilized by one of the usual methods. About this time an epidemic of scurvy broke out in Berlin among some hundreds of infants who were receiving pasteurized milk supplied by one of the largest dairies. In our own country the matter was considered of sufficient importance to lead to the formation of several medical committees to inquire into the nature of this new disorder. After much discussion, the ailment was admitted to be true scurvy, differing from the disorder of adults, which had been recognized for hundreds of years, only by the fact that it developed in infants. It was found to vanish, as if by magic, on giving a few ounces of orange juice. Today every intelligent mother knows that a baby fed on cow's milk must receive an antiscorbutic supplement—orange, lemon or tomato juice.

Infantile scurvy may be dormant for many months. Disordered nutrition develops far more frequently from a lack of an essential food factor than the stereotyped nutritional disorders, such as scurvy, beriberi, pellagra and xerophthalmia. However, these mild forms of dis-

turbed nutrition are merely abortive manifestations of one of the deficiency diseases. The baby may become pale, lose its appetite and cease to gain. This condition has been termed "latent scurvy," and is more to be feared today than the florid, acute form. It occurs sporadically among infants of the well-to-do; how common it is we have no means of determining. It is this form of scurvy that will be eradicated as the result of our fuller knowledge of the specific vitamin, and our ability to assay it quantitatively in the various foods in which it is contained.

Vitamin C and Scurvy.—Recently scurvy has acquired an entirely new interest, as the classical representative of a group of disorders which has revealed the essential importance to man of hitherto unknown dietary factors—the vitamins. Although we do not yet know the exact chemical nature of the antiscorbutic vitamin, we have learned from laboratory experiments that the welfare and even the life of almost all animals is not possible without the inclusion in the dietary of a small quantity of this food factor. As has been so often the case, a great advance in our knowledge and understanding has immediately followed our ability to induce a disorder at will in an experimental animal. Today we can control the factors which lead to the development of scurvy, and measure the protective substances quantitatively so that we can either ward it off or gauge the extent to which we would have it develop. Such knowledge has greatly changed our ideas as to the antiscorbutic value of various foods and more particularly of the effect of processes to which they are subjected. Before we thought of foods in terms of vitamins, it was believed that if milk, fruit or vegetables were

subjected to heat they lost their power to protect against or to cure scurvy. It should be added, however, that the rôle of heat in bringing about this destruction in milk was questioned by numerous experienced and reliable physicians who claimed that they had fed thousands of infants solely on boiled or sterilized milk without the development of a single case of scurvy. The clinical accounts of these physicians were difficult to interpret, but the number of cases which they reported was too large to be disregarded. Since, however, we have an experimental method at hand by which each problem can be tested, this mooted question and many others have been clarified. As happens so often, it has been found that both parties were right.

The Vitamin Destroyed by Oxidation.—It is not the heat which mainly destroys the antiscorbutic vitamin but the oxidation which generally accompanies the heating process. If milk is heated in a vacuum, or if it is subjected to but little air during the course of sterilization, it preserves almost all of its antiscorbutic vitamin. On the other hand, if the access of air is free, as in the processes which brought about infantile scurvy in France and Switzerland at the beginning of this century, the milk loses almost all of this essential food factor. Catalysis greatly increases the velocity of oxidation and this furthers the destruction of this vitamin. A series of animal experiments demonstrated that when less than three parts per million of copper were added to milk, the antiscorbutic vitamin was appreciably reduced in the course of heating. Ageing, which in reality is oxidation, is another factor which tends to destroy the antiscorbutic potency of foods. The fact that stale milk, that old vegetables, that dried fruit no longer possess the

specific protective or curative value of the fresh vegetable or fruit is now known also to be dependent on this same process—oxidation. It is not, therefore, the drying, the heating or the ageing *per se* which does the damage. It has been found, for example, that dried milk, which might be regarded as the prototype of a food which had been deprived of its antiscorbutic vitamin—for it has been heated, dried and stored—contains this factor in almost undiminished amount owing to the fact that it has not been subjected to oxidation.

Antiscorbutic Foods Rich in Vitamin C.—As the result of biologic tests it has been found that certain foods do not deserve their reputation as antiscorbutics, whereas others have assumed an important rôle in this respect. For example, lime juice, which for years was supplied to sailing vessels ("limers") to protect the sailors from scurvy, has been proved to contain but little antiscorbutic vitamin; its reputation has depended on the value of lemon juice with which it has been confused. On the other hand, tomato juice is now recognized as an antiscorbutic and is widely used in infant feeding. Furthermore, tests have proved without question of doubt—and that is the important contribution of the biologic method—that tomatoes lose but little of their specific value as the result of the twofold heating to which they are subjected in the course of the canning process.

These interesting conclusions, which have been deduced from work carried out in various countries during the past ten years, have a special significance in regard to the future of scurvy. With an exact knowledge of the amounts of the antiscorbutic vitamin contained in the various foods, and more particularly in

view of our knowledge of the agencies which tend to diminish this vitamin, it is certain that scurvy will occur to an ever less extent. Care will be exercised in carrying out industrial processes to the end that the antiscorbutic vitamin be not lost. Such precautions will be practiced partly because of definite knowledge in regard to the factors which tend to destroy this vitamin and also because we are in possession of an exact method for determining whether or not fruits and vegetables which are marketed have been robbed of their native and distinctive food value.

The disappearance of scurvy is to be attributed largely to empirical observations which were painstakingly garnered in the course of centuries and belatedly applied to our dietary. Its complete disappearance as a nutritional disorder in peace times will follow our newer and fuller understanding of the chemical characteristics of the specific vitamin upon which its development depends. As the result of this knowledge, we have recently learned how to preserve vegetables and fruits so that they retain their antiscorbutic properties throughout the winter season. It is not too much to hope that the day is not far distant when a method will be devised to elaborate the vitamin which protects against scurvy, as we have recently succeeded in doing in rickets.

Chapter V—Article 3

THE ADVANCE AGAINST PELLAGRA*

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Dr. Goldberger has been a medical officer of the United States Public Health Service since 1899 and since 1914 has been a director of its field studies in nutrition. In addition to his studies of several infectious diseases he has achieved distinction for his extensive investigations of the causes, prevention and treatment of pellagra which, largely through his researches, has been assigned to the category of dietary deficiency diseases.

It is now nearly two hundred years since the disease we know as pellagra was first recognized. Its cause has ever since been the subject of study, but, except for some of the secondary or contributing factors, authoritative opinion held, as recently as 1912 or 1913, that in any definite scientific sense its cause was unknown. Even the broad general question as to whether the disease was or was not an infection was unsettled, so that it may fairly be said that in all the long history of the disease our knowledge of its cause had, up to a dozen or so years ago, progressed but little beyond that of its earliest students.

In striking contrast is the advance registered as the result of the studies of the past ten or twelve years. The evidence now available seems to leave no reasonable doubt but that pellagra is caused by a faulty diet and that the primary dietary fault is a deficiency of a dietary essential or vitamin which has been designated by the symbol "P-P" (pellagra-preventive).

*Published with permission of the Surgeon General, U. S. Public Health Service.

It will be interesting, therefore, to review, even though necessarily very briefly, some of the more outstanding of the studies that have contributed to bring about this great advance.

Pellagra and Poverty.—As has been indicated, some of the contributing factors in the causation of pellagra were recognized by the earliest students of the disease. Conspicuous among these factors were poverty and rural life. The disease was believed to occur almost exclusively among the rural, agricultural poor. This naturally gave rise to the question as to why well-to-do people and, still more, the poor of urban populations were exempt. What difference was there between the conditions associated with poverty in the slums of cities and those associated with poverty in rural dwellers? And to which or to what combination of any such differences could the difference in susceptibility to the disease be attributed?

Among the oldest and most persistent ideas relating to pellagra was the idea that there was some connection between the disease and diet, and it had been repeatedly suggested that it was to the better diet of the well-to-do that this class owed its exemption from the disease. With respect to the poor of cities, there was reason to believe that they were likely to have a more varied diet and thus to be better nourished than the very poor of rural populations, so that the exemption of the former as contrasted with the high susceptibility of the latter might also be explained as due to a more favorable situation with respect to diet. These and certain other considerations pointing to the possibility that diet might play an important—perhaps dominating—rôle in relation to pellagra suggested to workers of the United

States Public Health Service that it might be worth while to test the idea that those exempt from the disease owed their exemption to their "better" diet.

Pellagra and Diet.—Acting on this idea they carried out a study of the preventability of pellagra by means of diet during the period 1914 to 1917, for one year at an orphanage in South Carolina, for two years at each of two orphanages in Mississippi and for three years among a group of insane pellagrins in a section of the Georgia State Sanitarium. At each of these institutions pellagra had been highly prevalent for several years. For the purposes of the study the diet of each of the orphanages and of a section of the Sanitarium was modified, among other things, by increasing liberally the allowance of fresh meat and milk, care being taken at the same time to see that the inmates actually ate what was served, particularly the meat and milk. Although all other conditions continued as they had been, the disease completely disappeared from among those receiving the modified diet.

The significance of this outcome will be better appreciated when it is recalled that in the light of all experience a relatively very large number of recurrences of the disease was normally to have been expected. And that these would with reasonable certainty have occurred is emphasized by the experience of one of the institutions following a relapse to the old diet; within a period of three and one-half to nine and one-half months after the termination of the formal test and the resumption of the unmodified diet 40 per cent of a considerable group of inmates developed pellagra. The diet of the affected group was thereupon again modified by increasing the allowance of fresh meat and milk and

the disease again disappeared, not a single case being observed among those of this group during a subsequent period of observation of fourteen months. Clearly, then, the outcome of this test was in harmony with the working hypothesis on which it was based and indicated unmistakably that pellagra could be prevented by proper diet, a conclusion that was tested and its soundness confirmed by an experience of British observers with pellagra among Armenian refugees at Port Said during the World War.

Even before this experiment was carried out the possibility that diet played a dominating rôle in relation to the causation and prevention of pellagra was strengthened in the view of the Public Health Service workers on finding in the course of some preliminary studies of pellagra in prisons, asylums and orphanages that the diet of the class of inmates among whom pellagra was highly prevalent differed appreciably from that of the class among whom no pellagra occurred. It was found that the diet of the affected group differed from that of the exempt group most conspicuously in that it included much smaller quantities of fresh animal protein foods. Since one of the most conspicuous differences between the diet of the well-to-do and that of the poor is the difference in the quantity of the fresh animal protein foods (meat, milk, eggs) it was suspected that such diet was for some reason faulty and had induced the disease in the groups receiving it. If this suspicion were well-founded it seemed reasonable to suppose that it should be possible to induce the disease experimentally by feeding such a diet. An opportunity to carry out such an experiment at one of the Mississippi State Penitentiary farms was afforded these workers early in 1915 through

the cooperation of the then State Health Officer, Dr. E. H. Galloway, and the Governor, Earl Brewer. Twelve white male convicts offering themselves were furnished a diet consisting of dishes made from cornmeal, grits, white rice, patent wheat flour, sweet potatoes, sugar, syrup, greens and pork fat. Not less than six of the volunteers developed the disease in definitely recognizable form. There were forty-seven other residents at this penitentiary throughout the period of this experiment, but none of these developed any signs of the disease. This result clearly showed, therefore, that pellagra could be produced by diet, just as the test started a year earlier, and then still in progress, resulted in proof of the preventability of the disease by diet.

A Protein Defect Not the Cause of Pellagra.—The question which now naturally arose was: What was there in diet that played this controlling part in preventing and in causing the disease? It was a question that had many times before been asked and that had received an almost endless variety of answers, none of them altogether satisfying. Continuing their studies, the workers of the Public Health Service now gave increasing attention to this question, and by 1922 they reached the conclusion that if, as was then generally believed, all dietary factors essential in human nutrition were known, the dietary defect which was responsible for the production of pellagra must be related to the protein —that is, that some protein defect was the cause of the disease, since they believed that they had been able to exclude from this rôle all of the other known essentials.

If this conclusion were sound and pellagra was indeed caused by a defective protein mixture, then, these workers reasoned, it should be possible to prevent the disease

by correcting this defect. Acting on this reasoning they carried out an experiment in which they expected to prevent the disease by supplementing the diet with a considerable quantity of a relatively pure protein in the form of casein—a procedure that considerably improves the nutritive quality of the protein of the diet. Their expectation, however, was not realized. Although there were indications that the pellagrins benefited by the casein addition, the disease nevertheless recurred. Clearly, something other than protein, and thus something other than any of the theretofore recognized dietary essentials, was concerned in the prevention and thus in the causation of pellagra.

Pellagra Caused by a Deficiency in a Specific Vitamin, P-P.—That this inference was sound and, incidentally therefore, that account would thenceforth have to be taken of a new dietary essential is strongly supported by the results of further studies quite recently reported by the same investigators. These results show that apparently the same new dietary factor, which they designated as factor "P-P," is essential for the nutrition of the dog, and, unless still another new, and at present indistinguishable, factor exists closely associated with it, also for the rat. In the dog, deficiency of this factor leads to the development of the condition known to veterinarians as black tongue; in the rat, deficiency of this factor, or (to allow for a remote possibility) of one very closely associated with and at present indistinguishable from it, leads to a condition of malnutrition characterized by an eruption which in certain respects is strikingly like that of pellagra. It is highly probable, if not quite certain, therefore, that we have in the dog and in the

albino rat suitable experimental animals for the study of pellagra.

Although the recent advance in our knowledge of the cause and prevention of pellagra has been relatively very rapid, the probability that the dog and the rat can now be made to serve as experimental animals may be expected to speed progress more than ever before. This is very fortunate, for there is still much to learn that is of fundamental importance in relation to pellagra and to its analogue in the dog, black tongue, and thus, indirectly, in relation to the broad general problem of animal nutrition.

As the result of the recent advance in knowledge the problem of pellagra has become more distinctively than ever one for the chemist and for the chemical pathologist. The factor "P-P" must be isolated and synthesized; its relation to the other nutritional factors and its distribution in foods must be determined. The chemical pathology of "P-P" deficiency is virtually a virgin field. All this is calling invitingly to properly equipped investigators. Thus there is good reason to believe that we are at the zero hour of a fresh advance in this one of the vital sectors of the battle line for knowledge in the "chemistry of life", nutrition.

Chapter V—Article 4

THE NEEDLESS SACRIFICE TO BERIBERI*

EDWARD B. VEDDER, M.D.

Lieutenant-Colonel, Medical Corps, United States Army

Lieutenant-Colonel Vedder has been in the medical service of the U. S. Army since 1903. Serving in the Philippine Islands he became interested in beriberi, of wide occurrence at that time in the Philippines, and one of the diseases earliest investigated which have been found to be due to deficiencies in diet. He is at present determining what grades of rice will prevent berberi. This work will probably eventually result in the elimination of beriberi from any country which adopts the standard he has set up. Lieut-Colonel Vedder is the author of "*The Medical Aspects of Chemical Warfare*" and is one of a group of distinguished investigators of the medical corps of the U. S. Army.

Beriberi is a disease that is hardly known even by name in the United States, although a few sporadic outbreaks have occurred in certain of our jails and insane asylums. But in the countries where it is endemic, beriberi causes an enormous total of morbidity and a considerable mortality. There are three principal foci of the disease, situated in Asia, Africa and South America. The Asiatic focus is most extensive and includes Japan, China, India, the Philippines, the Dutch Indies and the Malay States, or all the countries where rice forms the staple food. In South America beriberi occurs on the northern and eastern coast, especially in Brazil.

Statistics cannot be given for all these countries, but a very good idea of the extent of the disease in the Philippines may be gained from the following table

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from the Bulletin of the Philippine Health Service, November, 1925, Vol. 5, p. 596.

MORTALITY FROM BERIBERI IN THE PHILIPPINES

Year	Manila	Provinces	Total	Year	Manila	Provinces	Total
1910	1,441	4,128	5,569	1918	731	11,866	12,597
1911	1,331	4,367	5,698	1919	406	11,981	12,387
1912	1,056	4,372	5,428	1920	555	12,481	13,036
1913	696	3,194	3,890	1921	705	15,311	16,016
1914	838	4,102	4,940	1922	648	16,241	16,889
1915	872	4,336	5,208	1923	698	17,417	18,115
1916	684	5,874	6,558	1924	600	18,331	18,931
1917	490	7,463	7,953				

Since the mortality from beriberi does not usually exceed about 5 per cent of those affected, the enormous total of cases in the Philippines may be readily estimated. Beriberi is quite as serious a problem in China, Japan, and the Malay States.

Since beriberi is directly caused by an improper diet and can be prevented entirely by a proper diet, all the suffering, death and economic loss which beriberi causes may well be called a needless sacrifice.

There is probably no disease concerning which there is nothing further to be learned; the beriberi is no exception to this rule. But the problems that remain to be solved are chiefly scientific; for example, the accurate chemical identification of the beriberi vitamin or vitamins, and an explanation of the rôle that these substances play in the normal physiologic processes. We already know what changes in diet must be adopted to eradicate the disease, and future progress depends upon dissemination of this information and upon perfection

of administrative measures by the governments concerned. These are broad statements, and to substantiate them a brief discussion of what beriberi is and its etiology becomes necessary.

What is Beriberi?—Beriberi is a disease resulting from faulty metabolism and is produced by the deficiency of certain vitamins in the diet. This deficiency is produced usually by the too exclusive use of rice that has been completely milled, thus removing the external layers of the grain. It may be quite as easily produced by a one-sided diet of white wheat flour and other carbohydrate staples which are also deficient in these vitamins.

When such a deficient diet is initiated, no symptoms of beriberi are produced at once. But after a longer or shorter time, depending upon the intensity of the deprivation and the resistance of the individual, the symptoms of beriberi appear. This time interval between the adoption of the deficient diet and the first observable symptoms of the disease has been called the depletion period. The minimum depletion period in man is ninety days, a fact determined by a human feeding experiment conducted by Fraser and Stanton.

Clinically, beriberi is characterized by degenerative changes in the nervous system, including a multiple peripheral neuritis, and in certain cases this pathological change in the nervous system is combined with generalized œdema or dropsy, with effusions of fluid into the peritoneal and the pleural cavities and into the pericardium. The heart also becomes affected, is often considerably enlarged, and sudden death from cardiac failure is common. This complete combination of pathological changes is seldom found in one individual, and hence it has been common to describe two forms of beriberi, the

dry and the wet. In the dry form the changes in the neuro-muscular system predominate and we find a great loss of power of certain groups of muscles or perhaps of all the muscles, which become shriveled and intensely painful to the touch. Such an individual has lost weight, may be barely able to hobble around with the aid of a stick and is obviously very miserable. On the other hand in wet beriberi the muscular changes are not so pronounced, or at any rate the atrophy of the muscles is concealed by the œdema and such a patient's limbs may appear well rounded or even considerably enlarged.

Nevertheless in some of these cases the dropsy suddenly disappears, leaving the limbs shriveled and showing that the changes in the neuro-muscular system are much the same as in the dry cases. That is, the two types, the dry and the wet, may coexist and such cases have been described as mixed.

It seems highly probable that although both the dry and wet types of the disease have been included under the name of beriberi because they so frequently coexist, there are actually two deficiencies. Deficiency in one vitamin produces the dry type and deficiency of the second vitamin produces the wet type, and when both deficiencies coexist we have the mixed form of beriberi. It must be admitted that the existence of this second vitamin that causes the wet type has not yet been demonstrated. But generalized œdema without much admixture with dry beriberi has been frequently observed when certain improper diets were used. The so-called war œdema, epidemic dropsy and certain cases occurring on ships are probably caused by the same deficiency that produces wet beriberi. It is to be hoped that further investigation will elucidate this point and identify

the missing constituent in the diet that produces wet beriberi.

The Cause of Beriberi: Lack of an Antineuritic Vitamin.—Rice, like wheat, must be husked before it can be used. A grain of rice after removal of the husk consists of three layers. The outer layer is very thin and has been called the pericarp. It contains a large part of the mineral constituents or inorganic salts of the grain. The middle layer has been called the aleurone layer. It is thicker and consists of cells filled with albumen and fat. The greater part of the fat and albumen of the grain are found in this layer. The innermost portion or kernel contains by far the greatest bulk of the grain, and is chiefly composed of cells packed with starch granules. The germ of the rice is located at one end of the whole grain.

In the preparation of rice for food, the original domestic method was to pound the rice in a mortar until it was completely husked, in which process a certain portion of the two outer layers of the grain was removed, but the removal of these layers was seldom complete. Rice so prepared seldom caused beriberi. This method is still used by many natives who live in the country and grow their own rice and pound it out from day to day as they require it. But with the development of urban life came the trade in rice on a large scale. Whole rice does not keep well. It is apt to mildew in a damp climate, and rapidly becomes infested with weevils, moths and various other insects. Modern milling apparatus was introduced which removed the outer layers of the grain completely, producing the white and polished rice which we commonly see in the United States. This rice keeps well and can be shipped all over

the world. It is perfectly safe to use in a properly mixed and varied diet, but produces beriberi when it forms the major portion of the diet as is usually the case among poor Oriental races, and beriberi has increased *pari passu* with the introduction of milling machinery. We call rice from which the outer layers have not been removed "undermilled rice"; and when the outer layers have been completely removed, it is called "overmilled rice".

Epidemiological observations widely separated in time and place have shown that beriberi appears where overmilled rice is used as the main staple of diet for any length of time and that these outbreaks disappear where undermilled rice is substituted, and that beriberi never appears when a sufficiently undermilled rice only is used.

Vordermann studied the incidence of beriberi in the prisons of Java in 1895, where he had statistics from 279,623 prisoners. He found that in 51 prisons supplied with overmilled rice, beriberi developed in the proportion of one case to each thirty-nine prisoners. On the other hand when undermilled rice was the staple food there was only one case to each ten thousand prisoners.

Braddon showed that beriberi was almost unknown among the Tamils, a race living in the Malay States among other inhabitants who suffered severely. The Tamils ate cured or parboiled rice. Such rice is parboiled before husking and subsequently dried. As the result of such treatment, the husk is easily removed, leaving the external layers of the grain intact. Many other similar instances could be given did space permit.

We need only state here one further illustration, the case of the Philippine Scouts. This is a body of native

troops in the Philippines, largely officered by Americans, varying in strength from five to seven thousand men. The ration supplied has always consisted chiefly of rice. Prior to 1910 they were supplied with the white, overmilled rice and beriberi was a serious cause of disability. Often there were as many as five hundred cases annually, approximately 10 per cent of the entire command.

In 1910, as the result of recommendations made by the United States Army Board for the Study of Tropical Diseases in Manila, undermilled rice was substituted. During this year there were only about fifty cases, and as the substitution became more effective, beriberi was completely eradicated. Undermilled rice has continued to be issued and beriberi has never reappeared, and at the present date is unknown as a cause of disability for Philippine Scouts, although the disease often occurs among their families who do not eat the rice supplied in the ration, and beriberi is endemic among the native population among whom the Scouts live.

It has similarly been shown that the addition to the deficient ration of a small amount of a number of varieties of beans and peas as well as certain nuts will prevent the development of beriberi, and it has been found that these articles of diet contain a rich supply of the antineuritic vitamin. Yeast is also very rich in this vitamin, and has been used as a cheap source for its extraction. It can hardly be recommended as an article of diet, since beans are quite as efficient and much more palatable.

Administrative Difficulties in the Eradication of Beriberi.—It might be supposed that if the suppression of beriberi is so simple the disease would long ago have

been eradicated in all of the countries where it is now endemic. It must be realized, however, that the sanitary control of beriberi involves a radical change in the food habits of these Oriental races. It is notoriously difficult to change the food habits of the most intelligent populations, and it is quite impossible when dealing with ignorant peoples who do not believe the facts above stated, and who are too poor to purchase a varied diet. The eradication of beriberi among such races can therefore only be attained by means of governmental action prohibiting the production or importation of the overmilled and beriberi-producing rice. This method was suggested as early as 1912, and has been under consideration by the governments concerned. The administration of such a law would depend upon some chemical determination or other legal standard by means of which beriberi-producing and beriberi-preventing rices could be identified. Unfortunately up to the present time no such standard has been found. Some years ago it was suggested by Fraser and Stanton that a standard of 0.4 per cent phosphorus pentoxide would be satisfactory. Most of the phosphorus of the grain is in the external layers, and although the beriberi-preventing vitamin itself contains no phosphorus it was believed that the percentage of phosphorus gave an indication of the degree to which the milling of the rice had been carried. Although this standard is generally satisfactory, a number of samples of beriberi-producing rice have been found containing 0.4 per cent of phosphorus or more; and therefore the percentage of phosphorus cannot be used as a legal standard. A number of investigators are at present working on this problem, and it

is to be hoped that a satisfactory legal standard of a beriberi-preventing rice may soon be found.

The ideal method would of course be to determine the amount of beriberi-preventing vitamin in each sample of rice submitted. This could be done if the beriberi-preventing vitamin were identified chemically and a simple chemical test devised to demonstrate its presence. It may therefore be interesting to see what progress has been made in this direction.

The Antineuritic Vitamin.—Eijkman found in 1890 that fowls fed exclusively upon overmilled rice developed a peculiar form of paralysis that was rapidly fatal, and which was analogous to beriberi in man. Birds fed upon whole rice did not develop polyneuritis gallinarum, as the disease was called, and the disease could be prevented or cured by adding to the overmilled rice the polishings that had been removed from the grain in the process of milling. This simple experiment showed that there was some substance essential to life in the polishings removed from the grain and provided an experimental animal for the study of beriberi.

Subsequent progress was rapid. Fraser and Stanton found that the active substance could be dissolved out of the polishings by water containing 0.3 per cent hydrochloric acid, and that it remained soluble when alcohol was added up to 50 per cent. Chamberlain and Vedder found that this substance could be extracted by water, by 90 per cent alcohol, that it was dialyzable, and that it was adsorbed by animal charcoal. A number of observers then attacked the problem. Funk obtained priority in publication in December, 1911, and focused attention upon his work by naming this substance *vitamine*. But the work of Funk, Edie, Evans, Moore, Simp-

son and Webster, Suzuki, Shimamura and Odake was all independent and their results were published at various dates in 1912. Briefly, these observers found that the vitamin that prevented polyneuritis gallinarum could be completely precipitated from its solution by phosphotungstic acid. The phosphotungstates were broken up by treatment with barium hydroxide, and the remaining solution could be reprecipitated by various other precipitants, including silver nitrate and picric acid. All of these observers obtained small quantities of a crystalline substance that, in doses of a few milligrams, cured birds suffering from polyneuritis. Funk gave a melting point of 233° C. and an empirical formula, $C_{17}H_{20}N_2O_7$, to this substance. He has since admitted that this formula was inaccurate. The other observers mentioned realized that the crystalline product obtained was impure, and that in efforts to purify it the active fraction was lost. But the work demonstrated beyond question that this substance called vitamin existed, and was an organic base more or less closely related to the pyrimidine group. Vedder and Williams also obtained this antineuritic vitamin in 1913, and indicated the probability that there was a second vitamin deficiency responsible for the wet type of beriberi.

The difficulty in recovering this antineuritic vitamin in any considerable amount lies in the fact that its presence or absence in any given solution can only be determined by a biological test on a bird suffering from polyneuritis, and in the additional fact that it is rapidly destroyed or rendered inactive by many of the most useful chemical methods. Thus it rapidly becomes inactive in alkaline solutions.

Seidell has experimented on the extraction and identification of this vitamin from yeast. The molecular weight of the base by his process has been given as 201, and a provisional formula of $C_8H_5O_3N_3$ has been obtained for it.

The antineuritic vitamin has therefore not yet been exactly identified in spite of persistent efforts during the past fourteen years. It is highly probable that it will soon be identified.

It has been very generally assumed that this antineuritic vitamin is the same as the vitamin called water-soluble B by McCollum. This may or may not be the case. The identity of the two can only be shown by knowing the chemical identity of both.

Seidell's method is far too complicated to serve as a legal standard for a beriberi-preventing rice, nor does it give any indication of the amount of vitamin originally present, since a considerable portion is lost during the extraction. Nevertheless if this vitamin is identified, possibly a simple chemical test can be devised to indicate the amount present in a given sample of rice. It is even possible that it might be synthesized in large quantities.

Thus it appears that for the control of beriberi among native races, governmental control of the milling and importation of rice is essential, and that effective legislation waits on further contributions by chemists.

It is hoped that this article may help to stimulate further interest in the problem.

CHAPTER VI

Chemical Regulators of the Body

ARTICLE 1

THE INTERNAL SECRETIONS

ROY GRAHAM HOSKINS, PH.D., M.D.

Director of Research, Memorial Foundation for Endocrine Research, Harvard University

Dr. Hoskins held important appointments in a number of medical schools, before he became professor and head of the department of physiology at Ohio State University (1920-27). His masterly work in the study of the internal secretions led to his recent appointment (1927) as Director of Research of the Memorial Foundation for Neuro-Endocrine Research at the Harvard Medical School.

THAT various organs of the body form chemical substances which are of importance as regulators of body functions is an idea long entertained. A naïve form of this conception is the belief held by various primitive peoples that one can increase the store of his own virtues by consuming various organs of his fellow man. Thus his courage is enhanced by eating the heart of his enemy. A somewhat less naïve aspect of the same view has long persisted in the belief that diseases of one sort or another are due to lack of the various chemical substances that are normally supplied by the different organs. It follows that the diseases in question are to be cured by supplying artificially the lacking substances. This method of treatment was prac-

ticed more or less systematically as early as the time of Hippocrates. It was employed by Celsus and Dioscorides. For example, wolf's liver was prescribed for hepatic disease, hare's brain for nervousness and fox's lung for respiratory disorders. The use of thyroid gland substance in the treatment of myxedema is a brilliantly successful modern example of the same method of treatment. This system of therapeutics is known as opotherapy.

Principles of Internal Secretion or Hormones.—The peculiar chemical substances that are used in opotherapy are known as internal secretions or hormones. These may be defined as substances produced in one part of the body and distributed by the blood or lymph to other parts, the structure or functions of which are thereby modified.

There is good reason for the belief that this is the primitive method of control by which the various parts of the body are kept working in harmony. The chief lines of evidence are: chemical control is found in the lowest forms of animals and even in man; the so-called vegetative functions such as digestion or growth are preeminently under chemical control; reproduction, likewise, with its numerous bodily and psychic adaptations, is strikingly dependent upon hormone factors. It is perhaps significant in this connection that hormones seem to be completely interchangeable from one species to another. Thus, it is immaterial whether insulin for human use be obtained from a fish or an ox. This would seem to indicate that the hormones have been common factors from a remote ancestral period.

Presumably every body cell contributes more or less characteristic substances, the metabolites, to the blood

stream. Moreover, certain more specific chemical substances are known, such as those involved in blood-clotting or in resistance to diseases. All such substances more or less fall within the definition of hormones. This term, however, is arbitrarily restricted to the specific products of certain special organs, formerly known as the ductless glands. The organs known or believed to secrete hormones are the pineal, pituitary, thyroid, parathyroid, thymus and adrenal glands, together with the pancreas, stomach, upper intestines and the sex glands. Possibly the spleen and liver should be included and the thymus excluded. Hormone functions have been claimed for numerous other parts of the body, such as the lymph glands, the kidneys and the heart, but the evidence adduced in favor of the claims is too unsubstantial at present to warrant discussion in this place.

From a chemical standpoint the internal secretions are doubly interesting. The formation of the hormones is primarily a chemical process and their use by the body involves a multitude of chemical problems.

Remarkable Potency of Hormones.—The hormones so far as known are relatively simple bodies, for the most part crystalline. The chief active principle of the female sex glands, however, seems to be lipoid. They withstand boiling and some of them at least are not destroyed by the digestive fluids to which they are exposed when taken by mouth. Considered as drugs, they are of remarkable potency. Epinephrine, the active principle of the adrenal medulla, can readily be detected by biological assay in dilution of 1:300,000,000. To state this fact in terms that can readily be visualized, to reduce an ounce of epinephrine crystals to the dilution

mentioned, the contents of forty miles of water carts, each holding 625 gallons and deployed 200 carts to the mile, would be required. Abel has isolated a substance from the neural lobe of the pituitary gland so potent that 5000 miles of such carts would be required to reduce one ounce to the undetectable point. Thyroxine, which is probably a true hormone from the thyroid gland, is likewise highly potent. One milligram of this substance is sufficient to increase the total oxidation of the resting adult human body by 2 per cent. It has been estimated by workers at the Mayo Clinic that the total amount of thyroxine in use in the body at any one time amounts to about one-fourth of a grain. The total amount needed in a year is only about three and a half grains. But this pinch of material spells all the difference between complete imbecility and normal health. Nothing definite is known concerning the potency of other hormones. As regards the parathyroids it is known that the glands are minute in size and that the symptoms following their removal are severe and prompt in appearing. Presumably, therefore, the potency of the parathyroid hormone is of the same general order as that of the other hormones mentioned. A highly active substance has been obtained from the ovary and placenta, but has not yet been sufficiently purified to permit any adequate judgment as to its potency beyond the fact that a few drops of impure extract is able to bring a newly weaned rat to sexual maturity in four days (thirty-six to eighty days would be normal).

Hormones as Catalysts or Accelerators.—Their high potency suggests that the hormones act as catalysts. The direct evidence, so far as it goes, bears out this supposition. Indeed Kendall has worked out the chem-

ical mechanism by which he believes the thyroxine molecule acts alternately as a hydrogen acceptor and reducing agent, thus facilitating oxidation in the body. Epinephrine also seems to act in a somewhat similar way as an oxidation catalyst. The writer, with Hunter, has published evidence that the activity of intestinal muscle, limited by oxygen deficiency, is improved by a trace of epinephrine.

Synthetic Hormones: Epinephrine and Thyroxine.—The chemical composition of epinephrine and thyroxine is definitely known. That has made possible their artificial preparation by synthesis. The hormones, so far as known, show optical rotation. Natural epinephrine turns the plane of polarized light to the left. The synthetic preparation made in the laboratory as first obtained consists of equal amounts of dextro- and levorotatory substance. The former has little potency; the mixed or "racemic" form therefore has only about half the potency of the natural epinephrine. However, by the application of one of Pasteur's classical methods for separating dextro- and levo-compounds, the pure levorotatory epinephrine has been prepared from the artificial product and is found to be fully as potent as the natural.

Some knowledge pointing toward the composition of insulin and post-pituitary secretions is also available. As regards the other hormones, information is scanty or completely lacking.

Relationships Between Endocrine Organs.—Much has been made of the relationship believed to exist among the various endocrine organs. Perhaps on no scientific topic has greater exuberance of imagination been shown and, with so little basis, been seriously ac-

cepted. It is frequently taken as a truism that all these organs are intricately bound together to make up a functional system, that any change in the activity of one immediately reverberates throughout the series, to be reflected, ultimately, in the functions of every cell in the body. Thus, in case of need, one gland can take the place of a weak companion and so maintain the body in health. Or, if one gland becomes exuberant in its activity, some other gland may step in and supply an antidote or a secretion that has a soothing influence on the obstreperous member. Not only in case of abnormal functioning is this mutual control exercised, but no less under ordinary conditions does each gland serve as its brother's keeper, insuring a correct balance of the activities of each. Numerous diagrammatic schemes have been drawn up to epitomize these assumed relationships. Many a diagnosis, no less elaborate than fanciful, has been based upon such schemes, and many tons of dried visceral debris have been consumed by trusting unfortunates in the hope of correcting obscure disorders of growth or function ascribed to "pluriglandular disorders."

When subjected to the cold test of modern statistical science, however, many of the intricate glandular interrelationships seem to be purely mythical. Hammett of the Wistar Institute has recently spent months in an exhaustive mathematical study of gland weights in relation to one another. He had an abundance of excellent material. Presumably any change in gland function, he assumed, would ordinarily be reflected in some degree of change in weight, just as the biceps of a blacksmith comes to differ from that of a ribbon salesman. While Hammett found a few valid correlations, for the most

part his evidence showed that each gland goes its independent way under only such control as that of other parts of the body. The adrenal glands, alone, were found consistently to show extensive interrelationships with other endocrine organs.

On the other hand, a few instances of interglandular control have been rather satisfactorily proved. Thus Cannon and his collaborators have brought forth evidence that when insulin from the pancreas appears in the blood stream in excess, the adrenal glands at once pour forth an antidote to protect the body from serious harm. Smith has shown that tadpoles deprived of their pituitary glands are unable to change into frogs. Such animals show inadequate development of their thyroid glands. When, however, extract of pituitary glands of cattle is given to the defective creatures their thyroids take on normal development and metamorphosis soon sets in. That the pituitary has some regulatory influence on the ovaries is also not without considerable evidence. The adrenals have a marked influence in some cases on the development of secondary sex characteristics, and hence, presumably, on the primary sex glands. From this point on, however, the evidence becomes more and more nebulous, leaving most of the other possible interrelationships yet within the realms of fascinating theory.

The Pitfalls of Gland Therapy in the Absence of Recognized Hormones.—Much of the present vogue of gland therapy is hit-or-miss groping in the dark. In case of the few gland extracts that have been adequately studied, both as to their chemistry and their exact influence on bodily functions, we are on firm ground. Some of the greatest triumphs of modern therapeutics have been won in this field. Hundreds of thousands of

lives have been saved or made worth living by insulin or by thyroid substance. But the field is one beset with remarkable difficulties. Pitfalls lurk at every step and the keenest scientific acumen is required both to secure valid data and to interpret their meaning after they have been secured. Careless credulity is in no field of science more inept nor until recently, at any event, has it been more in evidence. The difficulties of endocrine study in general can be illustrated by a consideration of the problems of gland therapeutics.

Final proof of the actual existence of any given hormone has proved most evasive. Logic demands that it be shown not only that deprivation of the gland in question exerts an influence on the body and that the gland produces some peculiar product during life, but that the peculiar substance is discharged as such by the gland into the circulation. Short of all these steps in proof, the possibility always remains that the gland in question functions by a method of subtraction from, rather than addition to, the blood stream. If the gland serves only to remove harmful substances, the entire basis for substitutive gland therapy drops out.

The manufacturer of gland products is thus largely working in Stygian darkness with raw material the essential nature of which is unknown. He is attempting to process ore without knowledge of the metal it contains, or even a certainty that it actually contains any metal at all. Even granted that a given organ forms an essential hormone, the amount present in a mass of gland substance at any one time may be so slight as to be quite beyond capture. That is to say, the hormone may not normally be stored in the gland at all, but discharged as rapidly as formed. Granting that signifi-

cant quantities of hormone are present in the living gland, it may be chemically so fragile as to be destroyed before the gland reaches the processing laboratory. Again, even though the gland substance reach the laboratory well charged with potent hormone, if the chemist in charge knows nothing of its true nature he has no guarantee that it may not be completely destroyed by any step taken in preparing the gland product for market. There is no little reason to suppose, for example, that vast amounts of ovarian material have been rendered inert (though sold and consumed) by the simple process of extracting the fat from them in order to prevent subsequent rancidity.

Need for Exacting Investigations.—All this to one with any knowledge of the history of science points unmistakably to the need for many more exacting researches on fundamentals. One class of enthusiasts, however, frankly demands that the Gordian knot be cut forthwith by subjecting all kinds of gland substances to the therapeutic test. In any case, they say, the final value of the product will have to be assayed in the "crucible of the clinic." In the meanwhile (as they sedulously avoid saying), much profit will accrue to the venders of the gland products. This plea ignores the fact that hundreds of futilities have successfully passed the therapeutic test. Horse chestnuts have successfully warded off untold attacks of rheumatism. "Wound salve" applied to the weapon inducing the hurt has "cured" many a lesion. "Perkins Tractors" "withdrew disease" from thousands of victims. Electronic vibrations at present are "curing" a host of disorders. The crucible of the clinic returns reliable assays only in the hands of keen, well-trained clinicians. In

the hands of such men a large part of the gland products now widely exploited has been found to be so much rubbish. But psychotherapy, in its thousands of ramifications, continues its triumphal march from one victory to another. Fortunately, most glandular debris is at least harmless and as psychotherapeutic agents animal glands have a certain impressiveness. *De gustibus non disputandum.*

Hormones and Body Development.—One of the most seductive fields for endocrine speculation is that of the relation of the gland factors to bodily development. The baby born with an inadequate thyroid gland is a misshapen, drooling little being with protruding tongue and abdomen. He becomes a stunted, bandy-legged imbecile. Deficiency in the secretion of the anterior lobe of the pituitary causes a child to become a diminutive dwarf—not an infant, but a man in miniature. Early overactivity of the same gland makes him a symmetrical giant. If the activity begins after puberty, well rounded development is no longer possible, but the resulting overgrowth takes place in only such parts of the body as are still susceptible to the influence of the hormone. The result is a large misshapen individual who seems to have reverted to a gorilla type. Overactivity of the adrenal cortex causes a marked accentuation of the masculine sex traits. This may take place in either the male or the female, causing in the one an accentuation of virility in its various manifestations, and in the other a transformation toward the opposite sex. The deep-voiced, coarse-featured bearded ladies of the circus side-shows are probably victims of this glandular mishap. These and other types of deviation from the normal in man have their counterpart, as Stockard

has pointed out, in different varieties of dogs. The Pekinese is a dwarf, the Great Dane a giant.

Hormones and Evolution.—Since gland changes can induce such marked variations in the individual, the question arises: What part have the glands played in the evolution of human types? Kieth has speculated ingeniously and engagingly on this topic. He points out the resemblance between the Bushman and the hypothyroid individual. The large-boned northern type suggests the hyperpituitary giant. Kieth believes that evolution has to a considerable extent worked primarily through hereditary variations in the endocrine glands. It only may be pointed out in this place that the theory has numerous discrepancies yet to meet and remains almost entirely unsupported by direct "field evidence." No one knows, as a matter of fact, whether the so-called hypothyroid racial type shows congenital thyroid deficiency or not.

Future Progress.—The aim of this chapter has been to afford merely a bird's-eye view of the endocrine field, to point out a few of the more prominent landmarks. That in this field the outstanding therapeutic triumph of our generation has occurred will perhaps be conceded by anyone who has seen the lifting of the pall of hopelessness from the life of the diabetic by the discovery of insulin. As a gift to human happiness this discovery has had few to equal it in the history of science. In the long run perhaps even more significant than the immediate use of insulin as a remedy for diabetes will be its use as a working chemical tool for the elucidation of the mysteries of body metabolism. Whereas diabetes slays its thousands, obscure metabolic disorders now slay their tens of thousands. More recently, the prepa-

ration of an active extract from the parathyroid glands has added another useful therapeutic agent and working tool, the significance of which remains largely to be determined. This substance has a profound influence on lime metabolism and hence will probably prove of value in a variety of bodily disorders in which this is an important factor. In certain cases of spasms or of body spasticity its remedial virtue has proved remarkable. As this chapter is being written, reports of its beneficial effects in lung tuberculosis are appearing. Studies of the female sex hormones have recently been greatly stimulated throughout the biological world by the discovery that an active substance can readily be obtained from the fluid that collects in the ovary prior to the completion and discharge of the ovum. This extract has a remarkable stimulating effect on development of the mammary glands and other reproductive structures. Its effect on the psychic functions is no less remarkable. Studies directed to the purification, identification and standardization of this hormone product are under way in numerous laboratories. Its value as a therapeutic agent remains yet largely to be determined. What it can accomplish in the gross will be worked out in the clinic. How its effects are produced, and hence detailed knowledge of when and how to use it, will depend largely on laboratory studies. Although detailed data are not yet available it may be said that studies on the growth-controlling hormone of the pituitary are well advanced and are being assiduously prosecuted.

In the earlier decades of its history endocrinology received its greatest contributions from the hands of practicing physicians. Some of the outstanding fundamental facts regarding the adrenal glands, as we know them

today, were determined in the middle of the last century by Addison in his study of the disease that bears his name. Although Schiff's earlier experimental observations, had they commanded adequate attention, might have proved very enlightening, it was actually the work of clinicians on goiter that opened up the field of thyroid physiology. Our first substantial knowledge regarding the functions of the pituitary came from Marie in connection with his observation on acromegaly. But as the years have gone on, clinical observations have become more and more obviously inadequate to afford the sort of fundamental data that are essential to an understanding of the force of the endocrine factors in the biologic processes. Lacking these data, the practical use of gland products is beset with bewildering failures. We are forced more and more to turn to animal experimentation. The history of the treatment of diabetes will stand forever as an unmistakable illustration of the way to medical progress. The disease has been known from the dawn of medical history. Generation after generation of the ablest physicians had expended their best thought on the problem. Its gross features, complications and sequelæ were well worked out. But each generation stood as helpless as its predecessors until the work of the clinician was supplemented by that of the experimental investigator. Then, and then only, was the true nature of the disease apprehended, and then only was its specific remedy found. Without the aid of the experimentalist we should still be in the dark ages and thousands now alive would be but memories.

The workers who are willing to devote their lives to medical research are few, to be numbered only by hundreds in a world populated by billions. Their work is

difficult and largely thankless. In the case of such facts it is a sad commentary upon human intelligence that a chapter of this sort must needs close with a plea to all true humanitarians not only to support medical investigations but even more to interpose their sane common sense to protect the handful of active workers from the meddlesome interference of that group of misguided sentimentalists who, in the name of mercy, would condemn all the generations yet unborn to remain the helpless victims of biological ignorance.

Chapter VI—Article 2

THE HORMONES OF THE SUPRARENAL GLANDS

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Dr. Abel is our most famous American in the field of study of the chemistry of life. Associated first with the University of Michigan as a professor in its medical school (1891-93), his life work has been done almost exclusively at Johns Hopkins University (1893 to the present date). He has long been a member of the National Academy of Sciences and has been widely honored, in our country, in Europe and in South America. His pioneer work on the isolation of epinephrine, the internal secretion of the adrenal glands which helps to regulate vital body functions, has been followed by outstanding results in purifying an active principle of the pituitary gland and in preparing pure, crystallized insulin. Many other fundamental contributions to medical knowledge have been made by him, of which we can mention here only the proof that amino acids are present in the blood and, with the cooperation of his pupils, the discovery of indicators for the test of kidney and liver functions (see the article by Dr. Rowntree). He is an indefatigable worker. While he has had the generous support of his University, it may be said that if he had not had to devote so much of his life to teaching and lecturing he might have accomplished still more in the way of scientific research and have thus done more for the benefit of humanity

The suprarenal capsules or adrenal glands are two small structures, yellowish in color, shaped like a cocked hat and fitting snugly on top of each kidney. They were discovered and described in 1563 by Eustachius, an Italian anatomist, famed for many other discoveries, among them the Eustachian tube (the passage connecting the upper pharynx with the middle ear), the thoracic duct and many other structures of our body. In adults the weight of each gland is about 4 grams. These organs

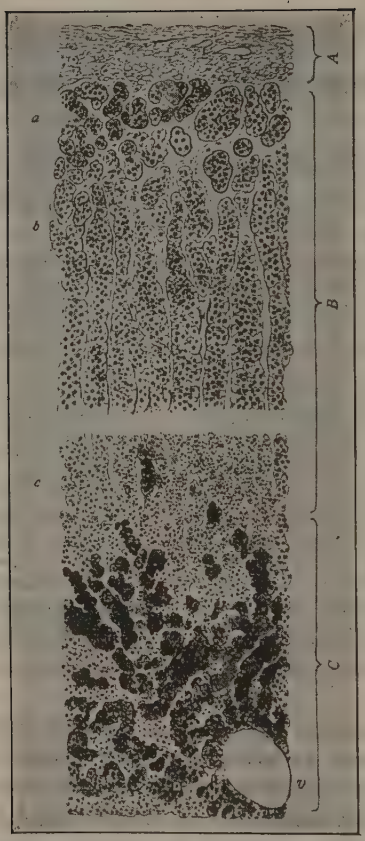


FIGURE 1. VERTICAL SECTION OF THE SUPRARENAL GLAND (MERKELHENLE). A. FIBROUS CAPSULE. B. CORTEX WITH THREE ZONES: *a*, GLOMERULAR ZONE; *b*, FACICULAR ZONE; *c*, RETICULAR ZONE. C. MEDULLA: v, VEIN IN MEDULLA (BAILEY).

are unique in having the richest blood supply of any of the structures in the animal organism—they receive a larger volume of blood per unit of weight and time than any other tissue in the body.

Structurally, the gland is made up of two portions which are quite distinct, embryologically, histologically and functionally. Epinephrine is derived from the medullary portion of the gland. (Figure 1.)

The stimulus for studying the physiological rôle of the suprarenal glands was initiated by Addison, who in 1855 published his clinical observations on "The Constitutional and Local Effects of the Disease of the Suprarenal Capsules." He noted in his clinic a class of patients with a very remarkable form of general anemia and with more pigmentation of the skin than is normal in northern Europe. He summarized the condition as follows:

> "The leading and characteristic features of the morbid state to which I would direct attention are anaemia, general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach and a peculiar change of colour of the skin occurring in connection with a diseased condition of the suprarenal capsules. . . . This singular dingy or dark coloration usually increases with the advance of the disease; the anaemia, languor, failure of appetite and feebleness of the heart become aggravated; a darkish streak usually appears on the commissure of the lips, the body wastes . . . the pulse becomes smaller and weaker, and without any special complaint of pain or uneasiness the patient at length gradually sinks and expires."

The above description gives the cardinal symptoms of this "by no means very rare" but almost invariably fatal malady, named, in honor of its discoverer, Addison's disease. After careful clinical and pathological investigations, Addison stated that he could find no proof

of organic changes anywhere in the body except in the suprarenal glands. At autopsy serious lesions in these organs, sometimes carcinomatous, sometimes tuberculous in character, were always found.

THE CORTICAL PORTION OF THE GLAND

Cortex Necessary for Life.—Within recent times the cortex of the suprarenal glands has been the subject of a considerable amount of research work, but despite this our knowledge in regard to the rôle of the cortex in the animal organism is still meager and inadequate, although we have several well-established facts to which reference will be made. About the time of Addison's published account of the disease which now bears his name, Brown-Séquard (1856) reported that the removal of both adrenal glands in animals caused them to die in from 24 to 48 hours. This fact has been confirmed subsequently by a large number of workers. Later Biedl studied certain fishes in which the cortex and medulla exist separately and found that it is the removal of the cortex which is responsible for the death of these animals. Subsequent investigators have substantiated these findings, and it is now generally accepted that removal of the cortex of the suprarenal usually leads to death, except in certain species of animals where the accessory cortical tissue is sufficient to take care of the physiological requirements of the animal.

A Hormone Produced in the Cortex.—Quite recently, Kuhn carried out a number of very significant experiments, from which he concludes that the cortex of the adrenal exerts the effect of a hormone or specific chemical principle. He bases his conclusions on the fact that

extracts of the suprarenal cortex, free from epinephrine, have a very decided effect in abolishing the symptoms of cortical insufficiency. Extracts made from other organs had no such ameliorating effects. Aub reported some preliminary studies in which an extract prepared from the cortex was efficacious in prolonging the life of adrenalectomized animals. Marine and Baumann have just published the results of their experiments dealing with the duration of life after suprarenalectomy in cats and an attempt to prolong the life of such animals by injections of glucose, glycerol and sodium salt. The authors conclude from their experiments that diuresis is one of the more important factors determining the duration of life. When the loss of water by diuresis is compensated for by an additional intake life is prolonged, and if not, life is shortened. These authors believe that all the manifestations of suprarenalectomy thus far observed are the individual details of a more fundamental and as yet unknown disturbance in nutrition in which the sympathetic nervous system is primarily concerned.

Some Physiological Effects of the Removal of Adrenals.—A number of studies have been carried out to determine the changes in the blood constituents following removal of the adrenals. Marshall and Davis first found a definite lowering of the excretory efficiency of the kidney in adrenalectomized cats, and, moreover, state that this may occur when such animals are apparently still in excellent condition and with a normal blood pressure. These findings led them to suggest a possible connection between the adrenals and renal activity. Their work has received confirmation from a number of investigators.

The cortex, according to Banting and Cairns, seems to act as a detoxicating agent for certain poisons derived from proteins; histamine, for example, is 30 times more toxic to dogs without adrenals than to normal animals, and dogs with a single cortex survive only until placed on a high protein diet. Dale first demonstrated this increased toxicity of histamine in adrenalectomized animals. The cells of the cortex are remarkably rich in lipoids and fats, and this fact has brought forward the suggestion that the cortex is intimately concerned with the manufacture of body lipoids, more particularly those found in the generative organs. The evidence for this is not as yet sufficiently convincing, and for the present it is perhaps best to leave the question an open one.

The Suprarenal Glands in Relation to Sexual Precocity.—From the studies of Hoskins and Hoskins and of McKinley and Fisher, there appears to be an intimate relationship between the cortex on the one hand and the reproductive system and growth on the other. These investigators found that feeding suprarenal glands to young rats caused a more rapid growth and development of the gonads than occurred in the controls. Sexual precocity is frequently found to be associated with tumors of the cortex. This abnormal condition is discovered every now and then in children of both sexes from four to six years of age, in whom the generative organs may be of adult size and function. It should be pointed out that sexual precocity, known technically as pubertas precox, may result not only from tumors of the suprarenal cortex but also from disturbances, usually tumors, in other glandular structures, such as the pineal and gonads, and from hyperplasia of the anterior portion of the pituitary body. There are not a few cases on record also

of handsome well-built young girls, with all the characteristics of their age and sex, who have had to undergo the distressful experience of what amounts to a change in sex: in medical language, they have ceased to develop as normal girls and have come to exhibit the symptoms of virilism. The condition of these unfortunates is due to the more or less slow growth of a tumor, often benign in character and, histologically speaking, consisting only of an overgrowth of the normal cortical tissue. The tumor leads to such an interaction with the sex glands and other endocrine organs that male characteristics now develop. There is an excessive growth of hair on the chin, lips and cheeks and elsewhere on the body surface as in the adult male; the hands, the muscles of the arms and lower limbs, the appearance of the buttocks and thighs are those of a young male; the breasts become atrophic, the uterus grows smaller, and other changes are noted that need not be given here. Suffice it to say that in some of these rare cases of virilism, the unfortunate victims of the disordered function of the suprarenal could be restored to their original feminine state by the surgical removal of the suprarenal cortical growth.

The weight of evidence seems to favor the view that the adrenal glands hypertrophy during pregnancy and lactation, and that during growth the cortex increases proportionally more than does the medulla. Tamura noted that in the mouse there is a hypertrophy of the medulla towards the end of pregnancy. The size of this gland increases slowly and reaches its maximum at about the third quarter of pregnancy, followed by a partial decrease during the last quarter.

The Need for Further Research.—It would seem from recent experimental work that we may expect in the near future significant developments and a clearing away of the obstacles to a better understanding of the significance and physiological rôle of the cortex in the animal. Certain it is that there is a specific chemical principle or hormone elaborated by the cortex which plays an indispensable rôle in the animal economy. The experimental results of numerous investigators make it necessary to regard the cortex and medulla as having separate functions, although the possibility of an interdependence of these functions must not be lost sight of.

THE MEDULLA AND ITS ACTIVE PRINCIPLE

Shortly after the announcement of Addison's discoveries (1856), a French physiologist, Vulpian, discovered that an extract made from the crushed suprarenal glands of animals and of human beings gave a striking emerald-green color on the addition of ferric chloride. No other tissue, either of the higher or lower animals, gives this reaction. These discoveries had no notable consequences until many years after, when, in 1894-95, Oliver and Schafer and, independently of them, Cybulski and Szymonowicz, demonstrated that suprarenal extract contains a substance which in minute concentration causes a remarkable rise, of brief duration, in the blood pressure, in consequence mainly of a powerful constrictor effect on the arterioles of the body. Investigators soon showed that the substance responsible for this vaso-constrictor effect is found only in the medulla of the suprarenal gland and not in its cortical portion. Subsequent researches showed that the substance causing the cardio-vascular changes is a chemical compound

which easily is oxidized on exposure to the air or on the addition of oxidizing agents in general, turning first pink, then brown, and finally yielding a dark amorphous substance, a so-called melanin. It is this principle also which, after oxidation, is responsible for the dark color of the medullary portion of suprarenal glands which, after removal from the body, have lain exposed to the air for some time. The ancient anatomists referred to this softened and darkened medullary portion of the glands as *atra bilis*, or black bile.

Isolation and Synthesis of the Active Principle or Hormone.—This substance, as we now know, is an amino alcohol of a relatively simple constitution, characterized by the chemical name dihydroxymethylaminoethylolbenzene, $(HO)_2C_6H_3CHOH.CH_2NH.CH_3$. It is this one, among the many constituents of the medulla, that gives Vulpian's color reaction with ferric chloride. This substance, like most of our powerful alkaloids, turns the plane of polarized light to the left. It is now made synthetically, and medicine is no longer dependent on the adrenals of the ox or the sheep for its manufacture. It is called by various names, as adrenin, suprarenin, adrenaline and epinephrine, the last of which, derived from ἐπί, upon, and νεφρός, the kidney, has been adopted as the official name for the hormone by the U. S. Pharmacopeia. The hormone was first prepared synthetically in its racemic form by Stolz in 1906, and this was separated into its optical isomers by Flächer in 1908, since which time the levo-rotatory form, identical with that produced by the cells of the suprarenal medulla, has been at the service of medicine.

Abel and Crawford in 1897 were the first to isolate the hormone in the form of an active sulphate of a

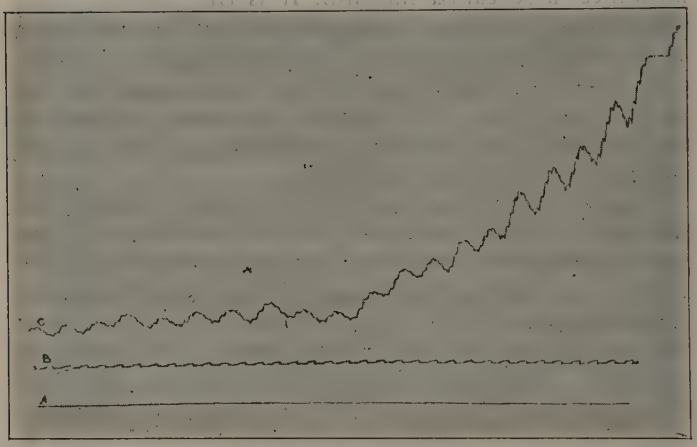


FIGURE 2. (EXPERIMENTAL RECORD PUBLISHED BY ABEL AND CRAWFORD IN JULY, 1897.) A. BASE LINE OF ARTERIAL PRESSURE. B. TIME, IN SECONDS. C. ARTERIAL BLOOD PRESSURE TRACING.

Dog anesthetized with morphine, curari and artificial respiration; both vagi cut. Injection at X of a highly active but amorphous sulphate of the adrenal hormone, obtained by saponification of the benzoyl derivative in which form the hormone had been separated from other medullary constituents. The tracing could not be continued because the arterial pressure had reached a point at which the mercury was driven out of the manometer. The pressure appears to rise more gradually than in curves 3 and 4 but this is due solely to the fact that in this early experiment a very rapidly moving drum was employed.

monobenzoyl derivative of the base (see *Johns Hopkins Bulletin,* No. 76, Vol. VIII, 1897). In Fig. 2 a blood pressure tracing is reproduced, taken on a rapidly revolving drum and showing the powerful vaso-constrictor action of the sulphate. Quantitative measurements of the effects of minimal doses of Abel's sulphate upon the arterial blood pressure of dogs were made by Reid Hunt in 1900, showing that as little as 0.083 millionths of a gram per kilo of body weight raised the arterial pressure 5 mm. of mercury. The effect increased *pari passu* with the dose until 5.7 millionths of a gram of the sulphate per kilo of body weight raised the blood pressure 66 mm. of mercury. These quantitative measurements compare not unfavorably with those made with equivalent amounts of the native unaltered base when the fact is borne in mind that Abel's sulphate had retained a single benzoyl group that resisted the saponification of the original compound (probably a tetrabenzoyl derivative of epinephrine) prepared from gland extracts (*Johns Hopkins Bulletin,* No. 128, Vol. XII, 1901).

This very reactive medullary hormone is characterized by a large variety of chemical reactions that can be used as qualitative tests for the base and some of which are well adapted for quantitative estimations. While these methods are quite serviceable in determining the amounts of epinephrine in solutions, they are not very satisfactory in certain physiological experiments, where the quantities of the active principle to be estimated are very small, as, for example, the minute amount present in a complex fluid like the blood. In such cases recourse is had to the intrinsically less accurate biological tests, such as the effect on the blood pressure of an animal

or on an intestinal or uterine strip of muscle. This latter is a very delicate method and allows one to detect very minute traces of the hormone. Another method is what is termed auto-assay. Here the blood is not withdrawn, but its content of epinephrine is estimated by reactions elicited in the experimental animal itself.

The Epinephrine Content of the Blood.—It is well established that this hormone is continuously being given off in almost infinitesimal amounts into the venous blood and thence distributed throughout the body in a very high dilution.

The figures for the epinephrine content of blood differ widely. This is not at all surprising, and probably indicates that the amount of epinephrine varies from time to time, depending on the state of the animal. That the output of epinephrine varies with circumstances is demonstrated by some recent experiments of Gley and Qumquand. The most recent investigation of the epinephrine content of the blood is that of Schlossmann, who could detect dilutions of epinephrine in serum to the extent of $1:10^{18}$. His method, however, is not suitable for the quantitative determination of epinephrine. For such purposes it is best to use the method of perfusing a rabbit's ear. Under normal conditions the epinephrine content of peripheral arterial blood is less than $1:10^{12}$, a concentration which probably is too low to have an ordinary vaso-constrictor or pressor action. After stimulating the adrenals, either by peritoneal irritation, sugar puncture or the injection of strychnine, the presence of epinephrine can be detected in the carotid blood, the concentration being below $1:10^{10}$. The hormone appears in the blood in a much higher concentration after a direct injection of nicotine into the

suprarenals or in asphyxia. In such cases its concentration in the peripheral blood rises to $2:10^8$.

The opinion that the medullary portion of the adrenal glands is not essential to life is generally held among investigators. This statement does not imply that the secretion from the medulla does not play a very important rôle within the animal organism. On the contrary, in the normal human being and in animals epinephrine, no doubt, does exercise a very definite function, as is clear from the work of Cannon and his associates and many others. It must be remembered, however, that it is very difficult to remove all traces of medullary tissue from an animal, and that may be the reason why investigators regard the medullary portion as being unessential to life, for the amount of tissue left may be sufficient to take care of the minimum physiological needs. Another possibility is that after removal of the medullary tissue the rôle of epinephrine may be taken over by another secretion, or other compensatory mechanisms come into play which enable the animal to carry on in an apparently normal way. It is known that the removal of one adrenal gland leads to a compensatory hypertrophy of the remaining gland and the animal is not visibly affected. Furthermore, removal of one gland and cutting of the nerve fibers to the other gland does not disturb the well-being of an animal, and outwardly the animal appears normal, it eats well and maintains its weight, but the behavior of such an animal to an injection of insulin has been profoundly altered; it has become very hypersensitive to insulin—only about one-fifth the normal dose will suffice to throw such an animal into violent convulsions. Hence, in interpreting the effects produced by the removal of a gland it is not

sufficient merely to observe the outward appearance and behavior of such a subject, for to all appearances it may be normal despite the fact that its metabolism may be very markedly altered in details, and yet, collectively, the results are about the same as before. The significance of the concept that the animal organism has a remarkable adaptive capacity in respect to the hormones, and that these may interact in the body in a mutually compensatory capacity or in an antagonistic manner, as the necessity arises, is just beginning to be appreciated as the manifold functions of the glands of internal secretion are being unravelled.

ACTION OF THE MEDULLARY HORMONE, EPINEPHRINE

Before discussing the many pharmacological actions induced by the injection of epinephrine or suprarenal extracts, it may be well to make clear that the type of action of the active principles of internal secreting glands, poured into the blood by the normal functioning of the glands, either continuously at a uniform rate, or at a reduced or increased rate to meet changing demands of the rest of the organism, may be quantitatively different from the action produced by injection of the isolated principles when these are introduced in relatively large amounts at one time and in a form perhaps not identical with the combinations in which they are naturally supplied by the glands.

We may next discuss briefly some of the extraordinary physiological actions that have been discovered in respect to this product in recent years. Foremost, there is a striking parallelism in the action of epinephrine and excitation of the sympathetic nervous system. In a word, epinephrine stimulates or inhibits the action of practi-

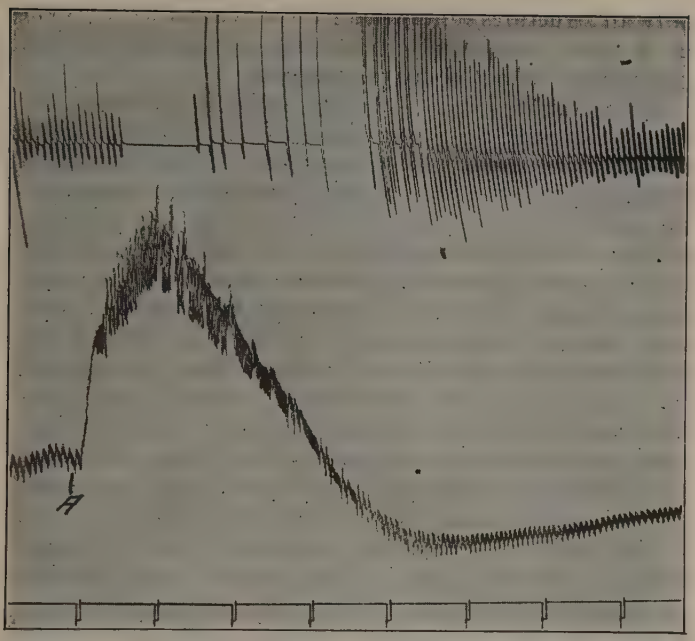


FIGURE 3. RESPIRATORY AND BLOOD PRESSURE TRACING OBTAINED FROM A DOG, WEIGHT 7 KILOS, ANESTHETIZED WITH AMYTAL. BOTH VAGI INTACT. UPPER TRACING—RESPIRATORY MOVEMENTS. MIDDLE TRACING — BLOOD PRESSURE (CURVE). LOWER TRACING, TIME IN HALF-MINUTES. AT THE PEAK OF THE CURVE THE VAGAL EFFECT IS MOST PRONOUNCED AS INDICATED BY THE SLOWING OF THE RATE AND AN INCREASE IN THE AMPLITUDE OF EACH BEAT. AT "A" 0.05 CC. OF A 1:1000 SOLUTION OF EPINEPHRINE WAS INJECTED INTRAVENOUSLY.

cally all structures innervated by the sympathetic system in a way analogous to electrical stimulation of the sympathetic nerves going to that part.

Action on the Heart.—For example, it accelerates the rate of the heart beat (a sympathetic reaction) in a way entirely comparable to that which follows electrical stimulation of the accelerator or sympathetic nerves to the heart.

The effect of epinephrine on the heart and circulation requires somewhat further discussion. In *very* minute doses epinephrine produces a fall in blood pressure, due to a stimulation of the dilator fibers in the sympathetic nervous system. In larger doses the effect on the blood pressure is a diametrically opposite one. This fact in itself is worthy of special notice, in that it illustrates the variation in the action of a drug due to the size of the dose employed. The effect on the heart and on the circulation of an average dose (0.05 cc. of a 1:1000 solution), when injected directly into the blood stream, is shown in Fig. 3. The stages of action may be discussed in three phases. During the first phase there is a sharp rise in blood pressure and an acceleration of the heart. The rise in pressure is due to a marked constriction of the blood vessels, including the capillaries, and also to an increased efficiency of the heart as a result of the stimulation of the sympathetic nerve which is the accelerator to the heart. In the second phase the heart rate is markedly slowed and the amplitude of each beat is increased. This is an indirect action connected with the rise of blood pressure, which acts as a stimulation of the vagus center, resulting in a slowing of the heart beat. That this is a vagal effect is shown in Fig. 4, in which the slowing effect has been abolished by cutting

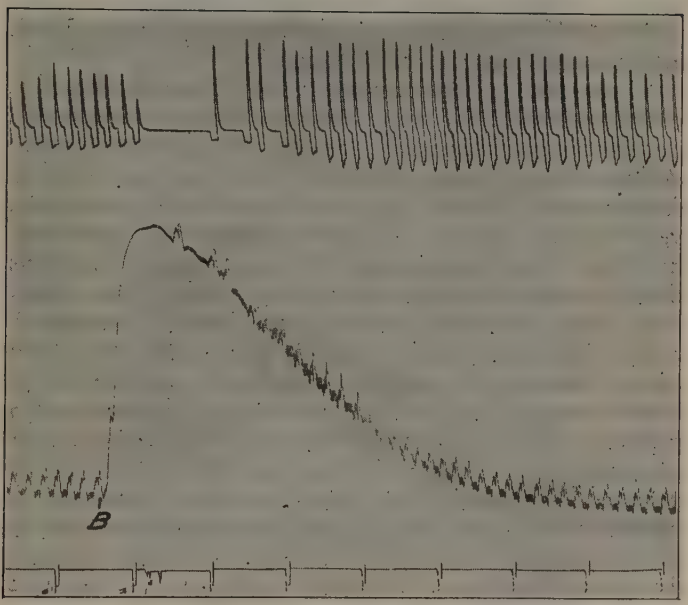


FIGURE 4. SAME ANIMAL AS USED IN FIGURE 3, EXCEPT THAT BOTH VAGI WERE SECTIONED BEFORE THE EPINEPHRINE (0.05 CC. OF A 1:1000 SOLUTION) WAS INJECTED AT B. NOTE THE ENTIRE ABSENCE OF VAGAL INFLUENCE AT THE PEAK OF THE BLOOD PRESSURE TRACING.

both vagi. A similar effect can be produced by injecting atropine, which paralyzes the vagal nerve endings in the heart. During the third, or recovery, phase the pressure falls rapidly and may even go below the normal level, and the heart rate becomes normal.

If the dose of epinephrine is very large the heart is overstimulated, as it were: the auricles and ventricles do not contract rhythmically and the ventricle fibrillates—*delirium cordis* being produced, and this may lead to sudden death. It may also be of interest to mention a very striking reversal of the blood pressure, discovered by Dale, when epinephrine is injected after a previous dose of ergot or, better, ergotamine, which is the chemically pure principle isolated from ergot. Tracings 5 and 6 show this very well. As stated and illustrated above, the injection of epinephrine causes a sharp rise of blood pressure. If, however, an animal has received a previous dose of ergotamine, then a subsequent dose of epinephrine will cause a fall instead of a rise of blood pressure. This reversal of blood pressure is thought to be due to the paralyzing of the constrictor terminals of the sympathetic nerves by the ergot principle, limiting the epinephrine now to its stimulant action on the unpoisoned dilator nerve terminals.

Epinephrine and the Respiration.—Epinephrine has also a very pronounced action on the respiration. The upper tracings in Figs. 3 and 4 show the respiratory changes. It will be noted that immediately following the injection the respiration ceases for a brief period—a period of apneia—followed by irregular breathing—periods of rest, alternating with periods of increased and deeper breathing. This action is of central origin. On the bronchial muscles epinephrine has a relaxing

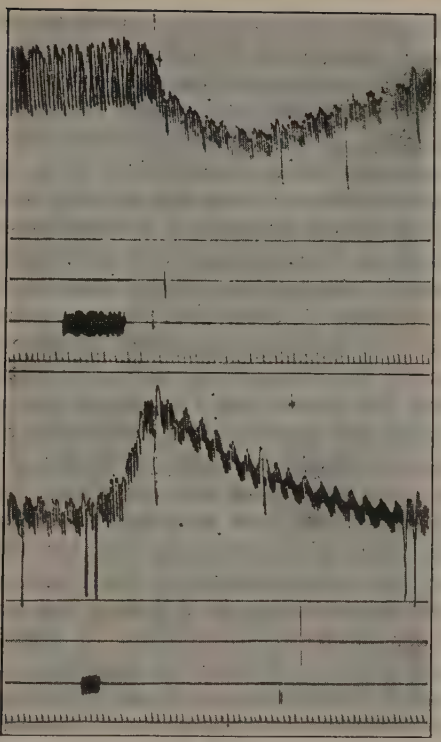


Fig. 5

Fig. 6

FIGURE 5. BLOOD PRESSURE TRACING SHOWING EFFECT OF INTRAVENOUS INJECTION OF 0.02 MG. EPINEPHRINE (ROTHLIN). FIGURE 6. BLOOD PRESSURE TRACING FROM THE SAME ANIMAL SHOWING THE FALL IN PRESSURE PRODUCED BY THE INJECTION OF 0.02 MG. OF EPINEPHRINE FOLLOWING A PREVIOUS DOSE OF 0.4 MG. PER KILO OF ERGOTAMINE TARTRATE (ROTHLIN).

effect. This important property is made use of in relieving asthmatic patients of bronchial spasm.

Other Effects.—In addition, epinephrine relaxes the stomach and intestines and causes increased tonus of their sphincters, as does stimulation of the splanchnic nerve. It causes a thick viscid saliva to be secreted, an increase in secretion of tears and of bile, a tremendous increase in the sweat secretion of some animals, as in the horse and sheep (but has no effect on this secretion in most animals), contraction of the muscles of the skin that raise the hair ("goose-flesh"), contraction of both the gravid and non-gravid uterus in many animals, relaxation of the non-gravid uterus in others, as the cat, the rat, the mouse and the guinea pig, contraction of the trigone and relaxation of the fundus of the bladder, relaxation of the bronchi, especially if they have previously been put into a state of spasm, dilatation of the pupil of the eye and protrusion of the eye-balls, conversion of the glycogen of the liver into blood sugar, increase of the red corpuscles of the blood and relaxation of the coronary vessels. All these reactions may be produced by electrical stimulation of the appropriate sympathetic nerve to the part in question.

A Drug of Extraordinary Potency.—It must be evident that we have in epinephrine a substance which must be classed in respect to its action and dosage with our most powerful alkaloids. It is, truly speaking, an autogenetic drug or hormone. And we are not surprised to learn that it has both antagonistic and synergistic relations not only to others of our hormones but to many alkaloids or other drugs, as ergotoxin, apocodeine, curare, atropine, physostigmine, histamine and the like. Naturally, a drug of such extraordinary potency and

such a variety of actions must always be used with care and only by those who are familiar with its capabilities. We are fortunate, indeed, that our suprarenal glands do not seem able, even in times of emergency, to throw out into the general circulation any but lower concentrations of this hormone. Life would be incompatible with the sudden liberation into the blood stream at any one moment of a considerable amount of it, say 15 to 20 mgs. The two suprarenals of an adult contain together at the most only nine milligrams of the principle. After acute infections and various exhausting diseases this amount is very much reduced, but on recovery regeneration of the substance seems to occur within a few days.

Insulin and Epinephrine.—The relation between the two hormones insulin and epinephrine has recently been studied by Cannon and his collaborators by means of new methods. In cats, under certain conditions, insulin increases the rate of the heart beat. As the sugar percentage continues to fall, the heart rate continues to rise, the increased rate going as high as 36 beats per minute. When the heart rate is augmented and other evidences of sympathetic activity are present, the injection of glucose intravenously is promptly followed by the disappearance of all these signs. This increased heart rate is explained as being due to a direct action of insulin on the adrenal medulla, in consequence of which an increased amount of epinephrine is poured into the blood stream.

Since the introduction of insulin, much discussion has arisen regarding the function of the suprarenal medulla in its possible relation to carbohydrate metabolism. This problem has been studied by Britton and

Geiling. After having carried out control observations on the response of normal dogs and cats to insulin, these investigators inactivated the suprarenal medullary tissues of the body insofar as this can be done. The operated animals maintained vigorous health but exhibited striking changes in susceptibility to insulin. Whereas the reactions due to too low a blood sugar value were commonly slight or absent on administering two or (sometimes) four units of insulin per kilo weight before operation, lower blood sugar values and convulsive seizures were often observed following the injection of 0.5 or (sometimes) 0.2 unit per kilo weight into the same animals after the suprarenal medulla had been rendered non-functional. The diminished secretion of the medullary hormone in these animals therefore resulted in a four- to ten-fold increase in their sensitivity to insulin. The animals showed no increase in blood sugar immediately following convulsions. That the glycogen reserves were adequate could be demonstrated, however, by the fact that small amounts of epinephrine rapidly elevated the blood sugar and promoted recovery. Corresponding increments in the glycemia did not occur on giving adrenaline to convulsant animals with suprarenals intact. The increased sensitivity to insulin of these medulli-suprarenal inactive animals has persisted for over three months. Complete protection against the lowering of the blood sugar percentage and insulin shock was nevertheless readily afforded by the administration at suitable intervals, commencing shortly after the administration of insulin, of small doses of epinephrine.

These experimental results of Britton and Geiling appear to show that the suprarenal hormone (epine-

phrine) is utilized in the normal animal as an inhibiting influence controlling to a certain extent the action of the pancreatic hormone insulin. This hitherto unrecognized but significant function is an illustration of the importance of the interrelations between the hormones of the body.

THE USES OF EPINEPHRINE IN PRACTICAL MEDICINE AND SURGERY

As was stated above, the official or pharmacopoeial name for the active principle of the suprarenals is epinephrine. The levo-rotatory form is fifteen times more active than the dextro- form and is the one generally used in practice. It is put up in the form of a 1:1000 acidulated solution and as an ointment, a suppository and an inhalant. The dried whole gland or whole gland extracts are not listed among the official preparations. Solutions of the pure active principle of the gland (l-epinephrine), made either from the gland or synthetically, have replaced in practice solutions made from the dried glands themselves. There are decided advantages in the use of the pure substance over the crude gland extracts. The solutions of the pure principle can be made up quantitatively, while gland extracts vary greatly in potency and are also liable to bacterial infection. It is not effective when administered by mouth and for that reason is usually injected subcutaneously and, rarely, intravenously.

Epinephrine has a number of important uses in medicine and surgery. The fact that it stimulates the sympathetic nervous system forms the basis for its employment in practical medicine.

Epinephrine in Bronchial Asthma.—Sufferers from bronchial asthma obtain almost instantaneous relief from the spasmodic labored breathing of the disease when they are given subcutaneously from 0.5 to 1 cc. of a 1:1000 solution of epinephrine. The relief is not permanent, and in severe cases the attacks may recur several times during a day and it may become necessary to give a little larger dose than that indicated above. But, fortunately for the patients, epinephrine can be used each time to bring about relief. No tolerance is developed towards epinephrine, so that it can be used as often as indicated. There are patients who have been victims of asthma for many years and who, no doubt, have had hundreds of injections of epinephrine, and still the drug is effective.

Epinephrine and Hives, etc.—In hives and serum sickness—clinical conditions thought to be due to hypersensitivity on the part of the patient to one or other foreign protein—epinephrine administered subcutaneously in doses of 0.5 to 1 cc. produces a very striking relief of the symptoms. In these conditions, too, epinephrine does not cure but it may be used as often as indicated.

The recent observations of Luckhardt and Koppanyi are of interest here. These workers noted that the effects of a subcutaneous injection of epinephrine could be very much enhanced and prolonged by massaging the site of injection, so much so that the physiological action of the drug can be extended over a period of hours.

Epinephrine an Aid in Heart Failure.—The fact that the sympathetic fibers are the accelerator nerves to the heart renders the use of epinephrine in certain cases

of cardiac failure or collapse particularly valuable. In some cases epinephrine may initiate contractions of a heart which has ceased to beat, for example, where the heart beat ceases during a surgical operation. In such instances it is advisable to inject the drug directly into the ventricle. A certain number of patients develop a condition of collapse due to an acute vascular paresis following the intravenous administration of arsphenamine; they exhibit the so-called nitritoid crisis. Epinephrine is injected into such persons as a prophylactic a short while before the arsphenamine is given. Epinephrine, too, is the drug of choice in cases where a patient is actually in such a state of collapse, for it constricts the blood vessels and thus counteracts the dilatation. The effect is fleeting and several injections may be required. Care, however, must be exercised that an overdose of the drug is not given, as it may have a deleterious action on the heart.

Epinephrine in Diabetes.—Epinephrine produces an increase in the sugar content of the blood, due to a mobilization of sugar from the liver glycogen, and for that reason it is used in diabetic patients who have taken an overdose of insulin with resultant lowering of their blood sugar to the toxic or convulsive level. In this emergency epinephrine can be of great service as a first restorative measure.

Epinephrine and Circulatory Weakness.—Dried suprarenal extract and epinephrine have been used therapeutically for purposes of substitution in patients with diseased or hypofunctioning adrenals. The results, viewed collectively, have been disappointing, although in a few instances improvement, usually only temporary, and, very rarely, a cure has been recorded. In condi-

tions of low blood pressure, debility and circulatory weakness, epinephrine has been reported as giving very good results, although it is by no means certain that its action in these cases is one of replacement therapy.

Epinephrine in Surgery.—Owing to the powerful vaso and capillary constrictor action of epinephrine, it is used very extensively in surgery to check surface bleeding. It is best used to control bleeding from mucous surfaces by local application and, where possible, together with packing of the bleeding part, such as in the nose and sinuses. It is considered inadvisable to use epinephrine for the purpose of checking internal hemorrhage, for in such cases the drug must be injected and then, while it will cause vaso-constriction, the blood pressure will be raised and this action is undesirable at such times. In operations performed under local anesthesia, epinephrine has proved of very great service. When mixed with the local anesthetic, such as cocaine or procaine, the rate of absorption of the drug is markedly reduced and at the same time the anesthetic effect is prolonged and intensified. Furthermore, the area to be operated upon is freed from congestion of blood. It is, however, well to point out that tissues with impaired circulation may heal more slowly, and in some few instances may necrose after the use of epinephrine, due to the vaso-constrictor effect. Care should also be observed not to use epinephrine on patients with exophthalmic goiter, because persons suffering from this disease are hypersensitive to this drug. Here is another example of the interrelation of the various hormones of the body.

Epinephrine and Shock.—Due to the fact that epinephrine raises the blood pressure and stimulates the

heart—both effects being brought about through stimulation of the sympathetic nervous system—its use has been suggested in conditions of surgical and traumatic shock. In such instances it is best to introduce the drug intravenously, with due regard to the dangers attending this mode of administration. At best, the beneficial effects, if any, in such cases are only temporary. In fact, the employment of drugs that raise the blood pressure (pituitary extracts and epinephrine) was discontinued in the treatment of shock during the late war. Much better results were obtained by the infusion of isotonic salt solutions, for thereby the circulation, especially in the capillaries, is improved, since that is the indicated therapeutic measure to institute. This procedure is also much more efficacious in the treatment of low blood pressure due to a sudden loss of blood, for in this condition also the essential thing to do is to restore the blood volume rather than reduce the circulatory bed by introducing vaso-constrictor drugs. In sudden collapse, such as may occur at times in connection with spinal anesthesia, the intravenous injection of epinephrine may be a life-saving measure. In cases of extreme emergency, epinephrine may be used to tide the patient over the interval before more effective measures can be instituted.

Chapter VI—Article 3

THE STORY OF THYROXINE

EDWARD C. KENDALL, Ph.D.

Mayo Foundation

Dr. Kendall has been the head of the section of chemistry of the Mayo Foundation, Rochester, Minnesota, since 1910. He was the first to isolate thyroxine, the active principle of the thyroid gland, which he describes in this paper. With Dr. Abel and other American investigators, he has contributed to keeping America in the foreground in the field of the chemical isolation of pure principles of secretion of our internal glands.

In the year 1895 especial interest was attracted to the study of the thyroid gland. Patients from whom this gland had been removed were greatly relieved by the administration of dried thyroid glands of animals, and when the thyroid glands of sheep or hogs were given in large amounts to normal human beings symptoms were produced which closely resembled those associated with some forms of goiter.

In addition to these observations, Baumann discovered that iodine was a constituent of the normal gland; by treating the glands of sheep with strong acid he was able to destroy most of the complex materials present and to separate a mixture of substances which contained about 9 per cent of iodine. This work was closely followed by an investigation of the iodine content of normal and diseased thyroid glands (goiters) and it became evident that the activity of the thyroid was closely related to the amount of iodine which the gland contained.

At about this time Magnus-Levy showed that the amount of energy produced by a patient suffering from

some types of goiter was almost twice as great as the amount of energy produced in a normal individual. It had been found that food and exercise increase the amount of energy produced by the body in a given time, but if a normal person is as quiet as possible and has not eaten food during the preceding twelve hours the amount of energy produced is remarkably constant. When this test is applied to people suffering from goiter or from lack of thyroid activity the amount of energy produced is not the same as in a normal person, and the severity of the disease may be judged from the rate of production of energy in the body. This test is known as the metabolic test, and since it is done without the influence of food or muscular work it is called the resting or basal metabolic rate.

All of these facts had been established prior to the year 1915. In this year a pure crystalline compound was isolated from the thyroid gland which contained 65 per cent of iodine, and experiments soon showed that this single white crystalline substance which was named thyroxine possessed marked physiological activity. One milligram, that is 1/66 of a grain of thyroxine, was found to increase the basal metabolic rate to the extent of 3 per cent, and furthermore a single administration increases the basal metabolic rate for a period of five to six weeks in a person who has little or no thyroid gland. Other experiments have shown that the total amount of thyroxine which is acting in the body is not more than 12 mg. or about 1/5 of one grain.

These results show that thyroxine must act as an agent which helps some chemical reaction to occur in the body and that it is not destroyed by this reaction. The reaction which it helps is undoubtedly intimately associated

Fig. 1 Fig. 2

EFFECT OF THYROXINE ON A CHILD SUFFERING FROM CRETINISM DUE TO DEFECTIVE THYROID SECRETION.

with the processes of combustion, and it probably acts as a catalyst. A catalyst familiar to many is the effect of manganese dioxide in some reactions. One of the first chemical experiments performed at school used to be the separation of oxygen from potassium chlorate. Since a high temperature is required before the reaction will take place with any speed, manganese dioxide was always added to assist the process. The manganese dioxide causes a prompt and rapid liberation of oxygen, although it is not consumed in the reaction. Without thyroxine, the chemical processes of the body will proceed slowly, and life be maintained sluggishly. The addition of thyroxine hastens the chemical reactions on which muscular and mental activity, glandular action, heart-beat and indeed life itself depend.

In addition to this influence on the basal metabolic rate it has been shown that thyroxine alone will relieve all of the symptoms in patients suffering from deficient thyroid in a manner just as satisfactory as will the dried glands of animals. There is also an influence on growth, and many other physiological processes in the body are stimulated by the administration of the thyroid gland of animals or by the use of thyroxine.

The illustrations, 1 and 2, of the effect of thyroxine on a child suffering from cretinism as the result of defective thyroxine secretion by her thyroid glands speak for themselves. The beneficial effect on the mentality of the child as well as on her growth and physical well-being should be noted. Illustrations 3 and 4 show the effect of the administration for thirteen months of the active constituent of the thyroid to an adult suffering from a deficiency in thyroid secretion (myxedema or Gull's disease). A diseased condition of the patient's thyroid

Fig. 3 Fig. 4

EFFECT OF THYROXINE ON AN ADULT SUFFERING FROM A DEFICIENCY IN THYROID SECRETION.

gland resulted in such an extensive destruction of the glandular substance that the required amount of the active principle from the gland was not produced. This condition can be shown to exist by a determination of the basal metabolic rate, which is the rate of oxidation of the body at rest.

While these results are of importance to patients suffering from disturbed thyroid function, they are also of interest to normal persons, because a human being is normal only when his thyroid gland is working, maintaining the normal basal metabolic rate and providing the body with a mechanism by which all physiological processes are kept under proper control. It is a study of these processes and mechanisms by which the body can convert sugar, fat, and protein into mechanical energy and heat which is most interesting to the research chemist. Through an investigation of these physiological processes we shall establish facts which will help greatly in our understanding of how the body not only produces heat, but also digests food and carries on the great mass of intricate chemical reactions involved in the complex human machine. It seems probable that the explanation of how thyroxine functions will serve as a type of a general reaction which is a common mechanism in the animal organism.

Within the last two years Dr. C. R. Harington in England has succeeded in preparing thyroxine artificially by synthesis from coal tar products. The process is a long one and may not result in a cheaper or easier process than the separation of this material from the thyroid glands of slaughtered hogs. However, the synthesis of this compound definitely establishes the chemical nature and exact configuration of this substance and is therefore

one more positive step forward in the progress of man's more complete knowledge of the chemical reactions which are essential for biological oxidations. Furthermore, the synthesis completely frees the investigator from dependence upon various forms of animal life, as now the entire synthesis of thyroxine can be carried out in the laboratory supplied with simple chemical reagents.

Chapter VI—Article 4

THE HORMONES OF THE PITUITARY SECRETIONS

E. M. K. GEILING, Ph.D., M.D.

Associate Professor of Pharmacology,
Johns Hopkins Medical School

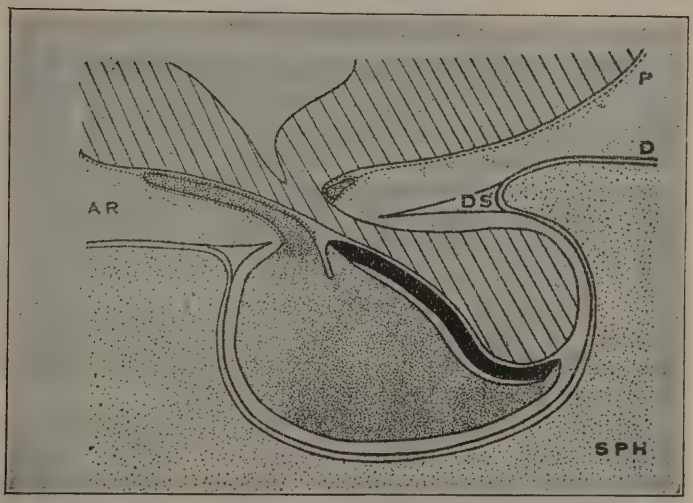
Dr. Geiling has been associated with Dr. Abel in the Johns Hopkins University Medical School since 1921. Previously he had been active in this field of work in South Africa. His most important investigations have been on the chemistry, function and interrelations of hormones.

The hypophysis cerebri, or the pituitary gland, a relatively small structure, weighing about 0.6 gram in man, lies in a groove at the base of the brain and is attached to the floor of the base of the brain by a stalk. The gland is made up of several distinct parts or lobes which differ in embryological origin and in histological structure. In addition the secretions or hormones formed by the cells in the various lobes differ in their chemical nature and in their physiological rôle within the animal organism.

There are certain structural differences in the glands of the various species of animals, but such variations cannot be discussed here. Suffice it to state that in man and in some of the higher animals the hypophysis is generally regarded as being composed of three lobes—anterior, intermediate and posterior—composing the gland proper which is attached by the stalk to the floor of the ventricle. The pars anterior is the largest lobe, possessing definite glandular cells, and has a rich blood

supply. The cells in this lobe elaborate a secretion which is directly taken up by the blood stream and transported to the different parts of the body. This hormone plays a very important part in the growth functions. The pars intermedia, the smallest portion of the gland, closely invests the posterior lobe, and extends up the stalk. It is composed of secreting cells, some of which dip in strands into the substance of the posterior lobe which is made up largely of neuroglia tissues poorly supplied with blood and lies upon, and is partly enveloped by, the anterior lobe. In addition to the three lobes just mentioned there is the pars tuberalis (Tilney), which is the term given to the thin epithelial layer which surrounds the *infundibulum* or stalk, and spreads out for a variable distance under the brain floor. Quite recently Atwell has published a communication in which he studied the development of the hypophysis cerebri in man with special reference to the pars tuberalis. The accompanying sketch taken from Atwell's paper gives a clear picture of the pituitary body and its surrounding membranes, the pia mater and dura, imbedded in the sphenoid bone.

At present it seems to be fairly definitely established that the so-called posterior lobe hormone (pituitrin, hypophysin, or pituitary liquid of commerce) is actually secreted by the cells of the pars intermedia which is intimately associated with the pars posterior and that the latter probably acts as a reservoir for the secretion. The route by which this "posterior lobe" hormone reaches the general circulation is still unsettled. Some investigators maintain (and the evidence in support of their contention is good) that the posterior lobe hormone passes into the third ventricle by way of the stalk



into the cerebro-spinal fluid and thence into the blood stream. Another group of workers believes that the secretion is taken up directly by the blood stream in the same way as is the anterior lobe hormone. It is exactly in the elucidation of such problems that a more thorough knowledge of the chemical nature of the hormones is exceedingly necessary.

The pars anterior of the hypophysis elaborates active principles chiefly concerned in general functions, and the pars intermedia cells form the posterior lobe secretion which, as will be shown later, possesses very powerful properties and also has an endocrine function. We may now discuss each of these lobes separately; a certain amount of overlapping, however, will inevitably occur, since certain experiments involve both lobes.

THE ANTERIOR LOBE

A Growth-Stimulating, Ovulation-Inhibiting Hormone.—Our knowledge in regard to this portion of the gland has been considerably advanced through the recent studies of Evans and his coworkers. These researches have been comprehensively reviewed in a recent Harvey Lecture delivered by Evans. The fact of the presence of a growth-stimulating and an ovulation-inhibiting hormone in the anterior lobe of the pituitary body seems to be established now with certainty. The evidence in support of this fact has been derived from various sources. Here we can discuss the results only of feeding experiments and of injections of extracts.

Injection of Extracts of the Anterior Lobe: Giantism.—Briefly stated, the results obtained by feeding experiments were very contradictory and the reason for it is now plain. Whole gland, or anterior lobe tissue, or extracts made from them, have been shown to be ineffective if taken by mouth. To produce their typical effects, extracts must be injected. Evans and his coworkers have produced typical giant rats by the daily intraperitoneal injection of anterior hypophyseal substance. In one instance a rat received 333 such daily injections, and at the end of that time it weighed 596 grams, whereas its healthy littler mate control weighed only 248 grams. Such injected rats show evidence of a certain degree of true giantism, and in addition there are very remarkable effects on the reproductive system. Oestrus or heat as detected by changes in the vaginal smear may never occur in these animals or may be exhibited only at long intervals. Hogben, independently, found that pituitary feeding has no influence on the

metamorphosis of medium-sized sexually mature Axolotl (Salamander) larvae, but injection of anterior lobe extracts into Axolotl of the same ages and dimensions was followed by the assumption of the adult characteristics with a rapidity comparable to metamorphosis by thyroid administration.

In regard to the chemical nature and properties of the anterior lobe hormone or hormones the available information is meager. Robertson prepared from the pars anterior of ox pituitaries by means of boiling alcohol and subsequent precipitation with dry ether a fraction named by him "Tethelin." This product, which no doubt is a mixture of substances, is claimed by him to have all the characteristic actions of the anterior lobe of the hypophysis when tested on the growth of white mice by feeding it to them. P. E. Smith, working with tadpoles whose pituitary had been removed, reports no influence on their growth by feeding tethelin. He finds that the growth-promoting substance is contained in the residual, alcohol-insoluble portion of the anterior lobe. Robertson in a rejoinder ascribes Smith's negative results to the fact that the tethelin was added to the water in a concentration insufficient to affect the growth of the tadpoles. Drummond and Cannan were unable to substantiate Robertson's findings. They state that tethelin is a very impure mixture of substances of the lipoid class. These conflicting observations illustrate the extraordinary difficulties met with in hormone investigations.

Evans and his associates prepared their extracts by washing the fresh anterior lobes of the hypophysis with 40 per cent alcohol, grinding them with sand in sterile physiological salt solution and centrifuging. The re-

moval of most of the proteins in these extracts was finally accomplished without destruction of the growth-stimulating or the ovulation-inhibiting properties.

THE POSTERIOR LOBE OF THE HYPOPHYSIS

It seems to be generally agreed that the "posterior lobe hormone or hormones" come from the pars intermedia, which contains secreting cells and which covers and in places dips into the posterior lobe which is largely made up of nervous tissue.

It is an accepted fact that the injection of extracts made from the posterior lobe of the hypophysis calls forth a very marked physiological response which manifests itself on the cardio-vascular, respiratory, renal, smooth muscle and certain glandular structures in the body, producing:

1. Sharp and sustained rise of the arterial blood pressure (except in the duck). Repeated injections have not only little or no blood pressure-raising action, but may produce a diametrically opposite effect—a depressor response. 2. The respiratory rate is quickened, but is interspersed with periods of cessation of breathing in unanesthetized dogs. 3. Diuretic-antidiuretic action. 4. Stimulation of the uterus, intestines, gall-bladder and urinary bladder; and dilation of the pupil. 5. Stimulation of secretion by the mammary gland, and an inhibitory action on the salivary gland.

In amphibians post-pituitary extracts also have a very pronounced action on the pigmentary system. Certain minor effects will be mentioned later.

It may be appropriate to emphasize here that the hormone of the posterior lobe of the hypophysis is an extremely delicate substance and very easily injured by

apparently mild chemical reagents. Owing to this fact it is certain that some investigators used partially injured material. Furthermore, aside from the active principle, post-pituitary extracts contain varying amounts of depressor bodies which may have a modifying action. The physiological effects of extracts depend, too, on the size of the dose. These facts and others to be mentioned are of value in enabling one to appreciate the number of seemingly divergent results obtained by numerous workers with extracts prepared in different ways (some undoubtedly injurious to the active principle) and used in very different concentrations and with a variety of animal species.

Chemical Principles in the Posterior Lobe of the Pituitary Body.—Numerous workers have carried out extensive studies to determine the number and nature of the active constituents of the posterior lobe of the hypophysis. These investigators were sharply divided in their opinions as to the number of principles present. All that can be done here is to summarize briefly the results and views of a few of the investigators. Thus, Dale and Dudley claim to have separated a principle which causes contraction of the smooth muscle of the uterus (oxytocic effect) from one that raises the blood pressure (pressor action). Abel and his coworkers were unable to accept these results. Hogben and Winton obtained evidence of a principle which acts on the melanophores or pigment cells and which seemed to be changed at the same rate as the oxytocic principle but much more slowly than the pressor principle, results which do not agree with the work of Dreyer and Clark and of Fenn. Quite recently Draper brought forth evidence in support of Dudley's conclusion.

These few selected instances must suffice to illustrate the conflict of results obtained in this difficult field. It should be noted that the investigators here cited, who stand for a multiplicity of posterior lobe principles, base their contention less on extensive methods of purification than on comparative physiological tests made with very crude extracts. Other investigators (Smith and McClosky, Leyko and Sikorski), on the basis of similar physiological experiments also made with unpurified extracts, came to the same conclusion as Abel and his associates that there is only one hormone in the posterior lobe.

Purification of the Hormone.—Abel and his coworkers made from the posterior lobe of the hypophysis a preparation in the form of a tartrate which far exceeded in potency any of the fractions obtained by the other investigators. While they did not claim entire chemical purity and individuality for their pituitary tartrate, they offered that this salt contains only one specific principle which possesses practically all the diverse physiological actions of a well-prepared aqueous extract.

This tartrate is extraordinarily potent as a smooth muscle stimulant, being 1000 to 1250 times more powerful than histamine phosphate on the isolated virgin guinea pig's uterus. In other words, maximum contraction of the uterus was secured in a dilution of pituitary tartrate of 1:15,000,000,000. In addition to this oxytocic action, this tartrate causes (1) prolonged rise in the arterial blood pressure; (2) a brief diuresis in the green-fed rabbit; (3) an antidiuretic action in diabetes insipidus; (4) no constriction of the bronchi of the dog (in large doses there is evidence of dilation);

and (5) a characteristic effect on the respiration of rabbits, as also on the breathing and the heart of unanesthetized dogs. These manifold actions, namely, oxytocic, pressor, diuretic-antidiuretic, respiratory and cardiac, are all obtained in high dilution with the purified tartrate. It should be mentioned in this connection that these investigators in the course of the development of their method of purification obtained preparations of varying degrees of activity, but in all cases there was found a striking parallelism in potency among the various physiological properties common to the pituitary tartrate.

Further evidence in support of the unitarian conception of the hormone in the posterior lobe of the hypophysis is seen in the following facts:*

(a) "All of the physiological properties of our active substance increase in intensity and in the same ratio *pari passu* with the methods of purification, from the initial precipitation of tartrate. It is unlikely that all of these physiological properties would retain their relative position to each other throughout a long series of varied chemical processes were they not characteristic of one and the same principle. (b) Exposure of the tartrate to normal sodium hydroxide for one hour at room temperature *completely destroys all* of its characteristic physiological actions. It is extremely unlikely that so comparatively mild a treatment as this should simultaneously destroy three or four separate principles. Guggenheim in 1914 was the first to show that alkali destroys both the pressor and the oxytocic actions of a pituitary extract, and held that this effect alone is sufficient to prove that both physiological actions are prop-

*Quoted from Dr. Abel's Harvey Lecture.

erties of one and the same substance. Since 1914 it has been shown by a number of investigators that every known physiological action of the posterior lobe is affected in a similar manner by exposure to alkali. (c) Boiling a solution of the tartrate with 1 per cent hydrochloric acid under a reflux condenser for one-half hour is also destructive of all these physiological properties with the exception of the oxytocic action, a certain proportion of which appears to withstand this short treatment with acid."

A number of investigators by entirely different procedures from those used by Abel and his collaborators came to the conclusion that the manifold physiological properties of the infundibular hormone are all inherent in one principle. Quite recently such evidence in support of the unitarian conception of this hormone was furnished by Professor Krogh, who has devised a new method of showing the presence of pituitary hormone in the circulating blood. By means of this technique he was able to show that the various physiological properties in different pituitary preparations have an approximately constant ratio.

Kamm, however, has recently announced (September, 1927, Meeting of the American Chemical Society at Detroit; Organic Symposium at Columbus, December, 1927), that, starting with a powder prepared from posterior lobes desiccated with acetone and whose oxytocic and pressor value had been carefully measured, he and his associates were able, by a process of extraction, salting out and fractional precipitation with organic solvents, to obtain two products, very similar in chemical properties, but one of which is highly oxytocic and free from pressor action while the other acts powerfully

on the blood pressure but has only a very slight action on the uterine muscle. Moreover, the total pressor and oxytocic action of the individual fractions are quantitatively the same as those of the original powder, showing that there has been no destruction of active principles during the process of separation. Preliminary diuretic experiments with the two substances indicate that it is the pressor principle which is responsible for the diuretic-antidiuretic action of pituitary extracts.*

In addition to the active principle there are depressor bodies present in the posterior lobe of the hypophysis (Schafer and Vincent). Abel and Nagayama have designated two of these blood pressure-lowering substances "B" and "C" from which they separated the active principle "A," which is the purified pituitary tartrate. The "C" substance is the well known chemical compound histamine and was isolated by Abel and Kubota in the form of a crystalline picrate.

Whether the effect on pigment cells belongs to the purified pituitary tartrate has not yet been established by Abel and his coworkers.

It is generally conceded that pituitary extracts do not exercise their optimum physiological action when administered by mouth, the reason being that the majority of investigators find that pituitary extracts are inacti-

*In the February, 1928, number of the *Journal of the American Chemical Society*, Kamm, Aldrich, Grote, Rowe and Bugbee published a paper entitled, "The Active Principles of the Posterior Lobe of the Pituitary Gland —I. The Demonstration of the Presence of Two Active Principles. II. The Separation of the Two Principles and their Concentration in the Form of Potent Solid Preparations." In this paper the complete experimental findings are presented in support of Kamm's statements mentioned in the text. Since the above mentioned paper appeared while this chapter was in press, we wish to refer the reader to the original as the work cannot be adequately reviewed in this footnote.

vated by tryptic but not by peptic digestion. In recent studies, however, Rees and Whitehead, who review the literature, conclude, on the basis of their experiments, that neither the gastric nor the intestinal enzymes destroy the action of infundibular extracts in raising the blood pressure and in causing the contraction of the uterus.

Interrelationship of the Pituitary Body with Other Endocrine Glands.—A fact of the utmost importance is that there exists a very close interdependence between the various glands of internal secretion; an excessive or diminished function of one of them is almost invariably reflected in one or more of the members of the endocrine system either in their structures or in the physiological functions controlled by the hormones elaborated by them. The facts are already many and their number is growing daily, and with each discovery new light is brought on this obscure but all-important phase of biology.

We may in conclusion refer to only a few of the numerous instances of the mutually antagonistic action of pituitrin and other glandular extracts. Thus, pituitary extracts inhibit the occurrence of excessive sugar in the blood following injections of epinephrine, and recently Burn, as also Moehlig and Ainslee, showed that the simultaneous injection of pituitary extracts and insulin into starved rabbits prevents the excessive exhaustion of sugar in the blood (hypoglycemia), which invariably follows from the injection of the latter substance alone.

Metabolism and the Pituitary Body.—Within the last year three monographs (deBois, King and McCann) on basal metabolism and an extensive review article by

Boothby and Sandiford have been published, and in them the relation of the pituitary gland to metabolism is discussed. Consequently here we shall take up only a few of the important points and attempt to give a summarized statement of the present status of our knowledge of the influence of the pituitary gland on metabolism.

The results of the careful and extensive studies by Benedict and Homans of the metabolic changes in dogs from which the pituitary had been removed are summarized under six headings—body weight, body temperature, pulse, respiration, total metabolism, development and general appearance. All these body processes are reduced, indicating a general lowering of all the normal body functions; some gain in weight, however, was noted, due to a deposition of fat. Winton and Hogben noted a lowering in the rate of carbon dioxide production in hypophysectomized frogs. These authors are inclined to attribute this to anterior lobe deficiency.

Other investigators have injected pituitary extracts from the different portions of the gland and obtained variable results. Weiss and Reiss studied the gaseous metabolism and increased utilization of carbohydrates after injection of pituitrin. A number of workers have made metabolism studies on normal human subjects injected with pituitary extracts, or on patients with various diseased conditions of the hypophysis, and it may be said that there is very considerable variation in the results obtained. This fact is not surprising if we consider the great difference in potency of commercial extracts, and that investigators did not all use the same dose. Furthermore, there must have been wide variations in the severity of the diseased conditions among the

patients studied. In general, it would seem that in many cases of excessive pituitarism there was an increase in the basal metabolic rate. Patients suffering from insufficient secretion by the pituitary gland show a condition of excessive obesity and a marked retardation of sexual development (Fröhlich's syndrome); in a number of patients treatment with pituitary extracts produced a lowering of the basal metabolism. The results, however, are by no means consistent. It is important to remember that there is a very striking interdependence between the various glands of internal secretion and that disturbances in metabolism in diseases associated with pathological conditions of the hypophysis may not necessarily be ascribable to that gland, but may be due to an alteration in the function of another gland (thyroid, or adrenals) resulting from a diseased pituitary body.

Pituitaryless animals are exceedingly sensitive to insulin. Recently Geiling, Campbell and Ishikawa have shown that one-twentieth of a normal dose of insulin for a dog will cause one of their hypophysectomized animals to go into violent convulsions. These investigators have further shown that removal of the anterior lobe alone will not render dogs sensitive to insulin, but removal of the posterior lobe, or rendering this portion of the gland inactive by sectioning the stalk or dislodging it from the blood supply will make an animal sensitive to insulin. These results of animal experimentation are of value in the guidance of physicians in insulin treatment of patients whose pituitary secretions may be below normal.

Methods of Standardization.—At present there are no chemical tests for either the anterior or the posterior

lobe hormone, which can be used for the quantitative estimation of these principles. Hence recourse must be had to biological methods. In view of the extraordinary potency of the hormones, standardization is absolutely necessary to render treatment of patients with pituitary extracts safe. At present two procedures are employed for evaluating the potency of posterior lobe extracts—the pituitary liquid, or pituitrin of commerce. The most delicate test is the action of post-pituitary extracts on a virgin guinea pig's uterus. For this purpose one horn of a virgin guinea pig's uterus is suspended in a bath containing Tyrode's solution, and one end is attached to a writing lever which records the uterine contractions on a smoked drum. The extent of the contractions produced by a given solution of unknown strength is compared with a solution made from a standard post-pituitary powder. The Hygienic Laboratory of the U. S. Public Health Service prepares from defatted pituitary glands this standard powder.

The second method of standardization of posterior lobe pituitary preparations is to compare a sample of unknown strength with a solution made from the standard powder by a study of the effect of small doses of each on the blood pressure of dogs.

CLINICAL USES

Blair Bell and Hick, and Hofbauer independently introduced pituitary extracts into clinical obstetrics. Since then this substance has found very wide use, and also much abuse, in obstetrical practice. Williams in a recent article gives the three main uses of this drug in practical obstetrics, namely:

(1) To stimulate contractions in the second stage of labor when the uterus is relatively relaxed, thereby hastening the completion of delivery, or obviating the necessity for application of forceps (Hofbauer); (2) to control hemorrhages following the extrusion of the placenta (Blair Bell); (3) to induce labor during the last three weeks of pregnancy (Hofbauer).

This writer adds further:

"It may be said that the use of pituitary extract in obstetrics should not be considered as harmless, since we are dealing with an extraordinarily potent agent, which is as yet imperfectly standardized. Generally speaking, I regard as somewhat dubious its use in the treatment of prolonged labor even under the most favorable conditions, and as extraordinarily dangerous in the presence of disproportion or of malposition of the child. Its greatest field of usefulness is in the prevention and control of atonic hemorrhage following the third stage of labor, while it is relatively efficient in the induction of labor during the last weeks of pregnancy."

Post-pituitary extracts are also used to allay post-operative abdominal distention by gas due to relaxation of the muscles of the bowel, a condition which many patients are wont to recall as the most painful part of their experience. In the treatment of diabetes insipidus also the extracts are very useful. A dose of 0.01 milligram (1/6500 grain) of the purest form of hormone (Abel's tartrate) applied in dilute solution for absorption in the nostrils promptly brings relief to the patient. The minuteness of the dosage offers further evidence of the remarkable potency of this chemical principle, which is apparent if we bear in mind that an average dose of strychnine, one of our most potent vegetable

drugs, is 1.5 milligrams (1/40 grain). A proportionate dose of the commercial pituitary extract is used in the treatment of diabetes insipidus.

Chapter VI—Article 5

HORMONES OF THE SEX GLANDS—WHAT THEY MEAN FOR GROWTH AND DEVELOPMENT

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Dr. Stockard has been on the staff of Cornell University Medical College, New York City, since 1906, holding the chair of anatomy since 1911. Specializing early in research in embryology, his life-long studies of embryonic development, of germ-cells and growth and development have made him a leading authority in this field. He is a member of the National Academy of Sciences and is associated with a number of important medical activities, such as the Rockefeller Institute for Medical Research and the Huntingdon Fund for Cancer Research.

The gonads or sex glands are extremely complex even as compared with other glands of internal secretion. This complexity is largely on account of the fact that the gonad is not only a gland of internal secretion but is also the reproductive organ of the individual and is, therefore, intimately involved in the entire sex problem. It is a point of some biological interest that the gonads are the only endocrine organs in the vertebrate which may be directly compared with a similar organ in the body of the invertebrate. All other vertebrate glands of internal secretion are either absent from the invertebrate body or have been homologized with most dissimilar structures. Although gonads are present in the two grand divisions of the animal kingdom, yet the gonads of the invertebrate seem comparable only to those of the vertebrate in being organs of reproduction or germ glands, there being no recognized internal secretion. The comparable parts

of the gonads in the vertebrate and invertebrate are simply, then, the germ glands, and there is actually no endocrine tissue in the invertebrate to be directly compared with the internally secreting cells of the vertebrate gonad. The internally secreting mechanism has apparently been added as a new part of the vertebrate gonad and we are as ignorant of its origin from the invertebrate body as we are of the origin of endocrine glands in general.

Effects of Hormones on Structural Development.—The hormones secreted by the gonads in higher vertebrates exert definite effects on structural development during three different periods of life. First: During embryonic development their secretions seem to regulate in a definite way the formation and growth of the primary internal and external genital organs. The proof of this statement is beautifully shown by the studies of Lillie and of Tandler on the development of the "freemartin" calf. When twins of opposite sex are produced in cattle it frequently happens that the arteries from the two placentæ become fused so that the blood from each embryo circulates through the body of the other. Under this condition the male calf develops normally but the development of the gonads becomes arrested in the female twin and the uterus, ovaducts and external genitalia of such a female individual are abnormal and poorly formed. The reproductive organs of the "freemartin" calf never recover from this early modification and the adult cow is defective and sterile.

After birth there seems to be little activity on the part of the gonads until just before the stage of puberty, then the second phase of gonadal effect begins. At the time of puberty sex-gland activity increases and the go-

nadal hormones become important factors in bringing about a fully completed expression of the secondary sex characters. The active secretion is necessary for the full development in the male of adult external genitalia and body hair and in the female for similar developments as well as for the mature growth of the mammary glands. If the gonads are destroyed by disease or removed before puberty these secondary sexual developments are never fully expressed.

Third: Following this heightened activity at puberty the gonadal secretions continue to be very important in influencing the general growth and development of the individual up to the state of complete adult size and form. There is evidence which would lead us to believe that the gonadal secretions actually influence head shape and the general body shape and height of the individual. It is well known, of course, that the eunuch does not present the typical shape and stature of a normal individual. Other well-recognized structural changes occur after menopause and during senility, which may be either primarily or indirectly associated with the disappearance of gonadal secretions.

In addition to this general life cycle of varying effects the endocrine tissues of the gonads also show a most pronounced short interval rhythmic activity. No other endocrine gland shows such well-marked rhythms. The secretory rhythm of the gonad, what one might style the chemical rhythm of the female, brings about a corresponding rhythm in the female genital tract and in reproductive activity. The rhythmical occurrence of ovulation in the female is associated with rhythmical changes in the secretions of the gonad.

A final important point of distinction between the endocrine tissues of the gonads and the other internally secreting glands is that the gonadal secretions seem more particularly associated with reproduction and the preservation of the species, while the other glands are more directly concerned with the preservation of the individual or self-preservation. Removal of the thyroid, adrenals or pancreas will result in great inconvenience or death to the individual, while the removal of gonads does not greatly handicap the individual's welfare, but completely does away with the possibility of reproduction.

These complex influences on growth and development and on the general sex rhythm furnish many varied points for attack in attempting to analyze the nature and play of the gonadal secretions. The study of these secretions is a broad, intricate biochemical problem that must be considered from many angles if it is to be properly analyzed.

Physiologically Proper Sterilization of Defectives, etc.— The commonly known glands of the body, such as sweat glands, oil glands, digestive glands, and so on, are supplied with a system of canals and ducts through which their secretions drain out upon a free surface. The salivary glands, for example, secrete saliva which flows out through ducts onto the surface of the mouth. The glands of internal secretion, on the other hand, are peculiar in that their products, the so-called hormones, are secreted into the small blood vessels and then circulate throughout the body. The ovaries and testicles, it must be remembered, are complex or mixed in this regard, since they have ducts for the passage of the ova and spermatozoa, while their hormones or internal secretions are not delivered into these ducts, but pass directly into

the blood capillaries and thus into the systemic circulation. This arrangement is of considerable practical importance, as it makes possible the separation of the problem of reproduction of progeny from the internal secretory influences of the gonads. It is possible, for example, to sterilize criminals and the mentally defective by tying off or obliterating the ducts leading from the testes to the urethra in the male or the oviducts or fallopian tubes leading from the ovaries to the uterus in the female without modifying the internal secretions of the gonads or changing the physical character or the sex behavior of the person. Such operations for preventing the reproduction of further defectives are thus from the physiological standpoint perfectly proper. On the other hand, the entire removal of the ovaries or testicles as a means of preventing reproduction not only does this, but immediately deprives the body of an important internal secretion which the gonads had supplied.

Effect of Sex Hormones on Personality and the Nervous System.—It is well known from our experience with castrated domestic animals that the disposition and mental attitude of the castrate is entirely different from that of the unoperated male. The castrated animal lacks pugnacity and determination in its behavior. The personality is actually changed, and this is due to the absence of the internal secretion from the testicle and not to the absence of reproductive cells, as may be deduced from observation on sterile individuals from which the germ cells are absent though the internal secretion from the gonad is still present.

The removal of any one of the internal secretions from the body brings about a new chemical constitution and often maladjustments in body function. The disturbed

state is frequently shown in a marked way by reactions of the nervous system. The extreme nervous tension of the menopause, or life change in women, psychical disturbance following the removal of the ovaries and the extreme changes in body shape and metabolism are well known phenomena. All such facts emphasize the need for isolation and chemical purification of the active principles of internal secretion. When such principles are available in pure form there is little doubt that many of our present ills will not only be better understood but actually controlled.

There is one fact regarding the action of hormones that goes far towards aiding and simplifying the task of isolation and chemical study. A hormone from a given gland of an individual is in no sense specific or characteristic of that individual or even of his race or species. The thyroid hormone from an ox or from a bird acts in the same way upon the tadpole of a frog as the thyroid of the frog itself would act. The active principles of the sex glands are probably the same in all vertebrate animals. Pézard as long ago as 1911 showed that injections of extracts from pig testicles would cause the comb and wattles to grow and develop in the caponized rooster. This observation has been confirmed by other workers, and such a reaction in the fowl might actually be used as an indicator for testing the efficacy of extracts from mammalian testicle.

Effects of Removal of Glands and Suppression of Secretion of Their Hormones.—We may now briefly review a number of general facts bearing on the nature and action of the gonadal secretion. The simplest means through which we have gained insight into the influence

of these secretions has been a study of the changes taking place after the removal of the glands.

There has been considerable investigation of the result of castration in human beings. The eunuchs of the East and the religious sect in Russia known as Scopec have been recently studied by Tandler and Gross and by Martin. The age at which castration is performed is a point of importance in the nature of the structural changes which follow. Typical body changes occur in both males and females after removal of the gonads. In all such individuals the thyroid becomes small and it is claimed that the hypophysis becomes enlarged, particularly in the female after ovariotomy. After castration in lower mammals there is a pronounced degeneration of the seminal vesicles and prostate and of the external genitalia. The human castrate grows tall, the shape of the pelvis is somewhat modified, and the general bone growth takes place as if reacting to a changed condition of growth regulation.

The removal of the gonads from birds produces changes quite comparable to those seen among mammals. In the case of the domestic fowl, the castrated capon shows a very reduced condition of its comb and wattles, while on the other hand the general plumage of such a bird is developed even beyond the gaudy male pattern. The general metabolism is changed and the body weight here increases as it does in the castrated mammal: thus, as is well known, the practice of castration is largely used for commercial purposes. When the ovary is removed from the hen she assumes after the next moult the plumage of a rooster and may grow spurs and actually behave in many ways like the male. If similar operations

be performed on ducks or other birds the results are very closely the same.

It is of considerable interest to note that the removal of gonads from the mammal causes both sexes to assume a state quite like the usual female type, while the removal of the gonads from birds, on the contrary, causes both sexes to develop plumage of the usual male type. In both groups, therefore, the characteristics of the operated individual approach in general the homozygous sex, which has in its cells the two X-chromosomes instead of one. In the mammals this homozygous two X-chromosome condition is in the female and among the birds it is in the male sex. This chromosome arrangement is supposed to be of prime importance in determining the sex of the individual at the time the egg is fertilized by the spermatozoon.

Among the invertebrates there are some beautiful examples of sex dimorphism, the male and female being widely different in both form and color, yet this seems entirely independent of the gonads. In some insects the male, for example, may have wing color entirely different from the female, or one sex may be wingless while the other is winged. The removal of the gonad even from the embryo of the invertebrate seems to have no effect whatever on the expression of either body form or color. It is, therefore, probable that there is no internal secretion from the gonad of the invertebrate comparable to that in the vertebrates, and here sex-dimorphism is regulated in a manner entirely different from that of the vertebrate.

Transplantation.—The action of the gonadal secretions has been extensively studied by transplantation experiments. We sometimes think of this as being a very

modern method, yet John Hunter one hundred and sixty-five years ago attempted to transplant the gonads in fowls and apparently succeeded in obtaining slight results. Recently this method has become very popular through the work of Steinach, Sands and many others. There have been feminization and masculination experiments in which certain body structures were made to change and resemble the opposite sex. Most of these modified characters are those which actually exist in both sexes, such as the mammary gland, hair, size, etc. Definite sex-limited structures such as the uterus and tubes in the female and the ducts of the male are not changed over toward the opposite sex. It would seem in general that sex determination in mammals at least seems to be definitely complete and the one sex cannot be changed into the other.

There have been many experiments on the effects of feeding with sex glands, but none of these have given clear or reliable results. This would seem to indicate that the secretions of the glands are not effective through the alimentary tract, at least in the forms administered. The injection of extracts has given more definite results. This again is not an ultra modern practice, since Brown Séquard in 1889 injected testicular extracts subcutaneously in man. He believed that effects were obtained. The present results from such injections are probably very little different from his—certainly we are hardly yet at a stage of definite practice on human beings. Much work of this nature has been done on lower animals. The results, however, have all been very indefinite, as might readily be expected since there has been no proper method for standardizing the gonadal extracts used either on

animals or the human being. This work must be followed to a stage of definite accuracy.

Secretory Cells or Tissues.—Since it seems so certain that both the testis and ovary actually secrete important hormones, the question arises as to exactly what cells or tissues in these organs are the important secretory elements.

The case of the testis is simpler than that of the ovary. Here the discussion has been as to whether the interstitial cells or the true generative cells produce the secretion. With a pertinent bearing on this question it has been found that a marked eunuchoid type might actually possess perfect spermatozoa while, on the other hand, a perfectly developed male type might have completely degenerate germinal epithelia. Such conditions as these make it seem that the interstitial cells are the important element in bringing about the fully expressed sex characteristics. The action of the embryonic secretion from the testis before there is any active germinal epithelium, as shown by Bissonnette in the "freemartin" studies, is also a case in point. X-ray treatments destroy the germinal epithelium at least temporarily without modifying the body type. The removal of most of the testicular material seems to have no effect on the body form—only a small remaining portion of one testis is apparently sufficient to maintain the normal male body condition. There is no hypertrophy of portions of the testis following the removal of a part.

The case of the ovary is more complex. It must not only influence growth conditions, but must later regulate uterine rhythm, placentation, and probably other phenomena. In the ovary a number of different kinds of cells have been thought to produce various secretions.

There are here interstitial cells believed by many to arise from atretic follicles, follicular cells, cells of the corpora lutea, and the ovum itself. It is very difficult, of course, to completely separate or isolate the activity of any one of these types in an experimental way. When one ovary is removed the other hypertrophies to a great extent and a small piece of ovary will grow into a whole organ. The single ovary tends to ovulate or give off eggs as abundantly or more so than both ovaries did originally. The ovarian secretions, as pointed out in considering the gonads in general, exert different effects on growth and development at different life periods. The ovary at menopause completely degenerates and yet the individual may live for years with no more rapid ageing after menopause than before the degeneration of the ovary. This fact in itself should make one question whether the lowering of the gonadal secretions is a primary cause of old age rather than one of the symptoms.

The secretions from the male and female gonads have been thought by many to be antagonistic in their actions. In order to test this, adult rats of opposite sex have been artificially grafted together, producing a condition of parabiosis. There was first thought to be antagonistic effects which caused the gonads of both components to become degenerate, but it was later found by several workers that a castrated animal might be fused to a normal animal of the same sex and yet the gonads of the normal animal would tend to degenerate under such conditions. Recently very interesting experiments have been made by Burns at Yale in grafting together tadpoles before the development and differentiation of sex, in order to determine whether one sex in its development might influence the other. It happened that every pair of

grafted tadpoles that lived to reach a stage where the sex could be identified were invariably a single-sex pair, either two males or two females. In this experiment, either all grafts of opposite sex have died, which seems somewhat probable, or else it might be that one component in the pair transforms the sex of the other so that both members of the pair are always of the same sex.

Effects of the Nervous System and of Other Endocrine Glands.—It should be remembered in considering all specific hormone effects produced by the gonads that other endocrine glands may very probably be involved. The nervous system is also concerned. The removal of eggs from the nest of a bird may cause a single female to produce as many eggs within forty days as she might have ovulated in five years. This is a definite effect on the ovary through the central nervous system of the bird in reacting to the taking away of her eggs from the nest. In line with this is the fact that menstruation and ovulation may be suppressed by hypnosis in the human female. Thus the central nervous system exerts a definite effect upon the hormone secretion of the gonads, and vice versa, the presence or absence of gonadal hormones exerts a marked influence on the physiology of the nervous system.

Hormones from other glands of internal secretion also play a rôle in regulating the behavior of the gonads. The effect of the removal of the thyroid gland on the ovary is well recognized by most physicians, and it is well known that among lower mammals a considerable degeneration of the ovary takes place after removal of the thyroid. Papanicolaou and Bagg in our laboratory have recently found that radium radiation on the thy-

roid in guinea pigs may cause a hyperactivity of this gland. The ovary in such animals gave a very marked reaction to this thyroid condition. Ovogenesis is greatly activated and the new cells migrate down into the stroma and form new Graafian follicles. There is also a change in the cellular picture of a smear made from the fluid of the vagina after radiation of the thyroid and the resulting stimulation of the ovary. This is quite an opposite reaction on the part of the ovary from that which follows the removal of the thyroid gland.

Need of Exact Research Work: Progress in Standardization.—All of the foregoing discussion shows the importance in the body economy of the endocrine secretions from the sex glands. Yet all of the phenomena so far discussed are simply qualitative changes resulting from a hit-or-miss method of experiment, and none of the results mentioned is definitely measurable or quantitative in type. This is necessarily true, since up to the present time there has been no definitely established indicator against which the gonadal secretions could be definitely measured or standardized. The results of experiments may be spectacular, but they are of little definite value unless quantitatively expressed and subject to accurate repetition. The chemical preparations or extracts of gonadal hormones must be brought up to some such point of standardization as that already obtained for the suprarenal substance and insulin. In order to attain this aim there must be devised some indicator against which these secretions may be accurately measured and tested. For the past eight years Dr. Papanicolaou and the present writer have been studying the ovarian reactions in guinea pigs by using the changes in the uterine and vaginal epithelia as an indicator for the ovarian condition.

The action of ovarian hormones on the uterus and vagina are very definite in giving certain cellular expressions which may be accurately recognized.

In making daily smears from the content of the vagina it was found that the cellular appearance changed in a very exact way in correspondence with the sexual stage of the animal. These observations were controlled by both operations and autopsies so as to establish a definite connection between the appearance of the smear, the condition of the vaginal wall, the uterine wall, and the ovary. These relations have been so clearly analyzed that anyone may examine a smear of the cells from the vagina and know very exactly what the condition of the uterus and ovary are at this time. By such a method one is actually able to predict within the hour the exact time of ovulation. These experiments have been extended by a number of other workers—Long and Evans with the rat, Allen with the mouse, Hartman with the opossum, and a number of others using different mammals. In all these animals it is readily possible to diagnose definitely the œstrous stages or sex periods and to know the exact state of the ovary from examination of the vaginal smear. It now becomes evident that any treatment or experiment which tends to influence the condition of the ovary might readily be detected by some modification in the vaginal smear. We have studied the effect of a number of modified conditions such as starvation and underfeeding and various operations, and found that in certain cases ovulation could be completely suppressed and the vaginal smear would definitely indicate the fact. If ovulation was accelerated this of course could be recognized in the same way. Allen by employing this method was able to detect the effect of the chemically

extracted follicular hormone on the uterine reaction of female mice from which the ovaries had previously been removed. In this way Allen and Doisey have learned many points of importance about the follicular hormone, and the possible means of its chemical separation.

A group in the laboratory at Cornell Medical College have been able to measure very definitely the quality and strength of several chemically fractioned ovarian preparations. There has been obtained a definite acceleration of ovulation in the guinea pig by injections of an ovarian hormone, and an equally clear-cut delay in ovulation as a result of measured tests of a purified lutean extract. Papanicolaou has recently reported in some detail these findings.

It seems quite evident from the amount of investigation already conducted that the vaginal smear method offers a most accurate indicator for the study of ovarian extracts and of ovarian reactions, and by the use of this indicator it is believed that the endocrine products of the ovary may be as accurately extracted and measured on the basis of unit doses as is now possible in the case of insulin. Certainly much work remains to be done, but it may clearly be followed along these lines.

An attempt to extend this work to human beings is now being made and through cooperation considerable progress has been attained. Dr. Papanicolaou finds that the various stages of the ovarian cycle in the human may be detected with considerable probability by examination of vaginal smears. The human smear is somewhat more difficult to analyze than that of the lower animals, but with experience it seems perfectly practical to use it as a general indicator. In working with the human cases it has also been found that early stages of pregnancy

and other abnormal conditions of the ovary and uterus may possibly be detected by characteristic changes in the nature of the vaginal smear. Papanicolaou has already reported in a brief way the general nature of the modified smear of pregnancy and his study is being actively continued at the present time. Much chemical investigation is necessary in order to understand the changes in the pregnant uterus which may modify the nature of the vaginal content.

Finally, we are encouraged to hope, in the light of the recent advances which have been made by the use of the vaginal smear method, that much accurate quantitative knowledge is soon to be obtained regarding the various functions of ovarian secretions. This method of study also furnishes a clear-cut means of detecting the influence of the secretions from other endocrine glands on the ovarian reaction. The relationships between the thyroid and pituitary and the ovary may be further elucidated by the present methods of study.

In concluding we may emphasize the remarkable fact that this histological indicator is as accurate and clean-cut in its response to chemical changes in the body as is any other type of diagnostic reaction, and it may aid in facilitating the more accurate chemical isolation of the gonadal hormones.

Chapter VI—Article 6

IODINE IN THE PREVENTION AND TREATMENT OF GOITER

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Dr. Marine was associate professor of experimental medicine at Western Reserve University (1914-20) before he was called to the directorship of the research laboratory of Montefiore Hospital in New York City. His studies of the incidence of goiter in school children, especially girls, carried out in Ohio in cooperation with Dr. Kimball, did much to awaken the world to the realization of the fact that iodine deficiency in food is the most common cause of ordinary goiter. The resulting word-wide movement to insure the presence of sufficient iodine in diets to prevent goiter is one of the greatest preventive measures undertaken by man in the interest of good health.

Our present knowledge of the importance of iodine in normal nutrition and its relation to disease, especially goiter, has been derived from so many sources that it seems of sufficient general interest to trace the development of the subject from its beginning.

Occurrence of Iodine in Nature.—The element iodine was discovered incidentally by Courtois, a saltpeter manufacturer of Paris, in 1811, and publicly announced in 1813. Courtois observed that the addition of sulphuric acid to the residue of seaweed ash after the extraction of soda caused a violet-colored vapor to be given off which crystallized in the cooler portions of the vessel in lustrous plates. Gay-Lussac and Sir Humphrey Davy gave this new element the name iodine (from ἰώδης,

meaning violaceous), since they recognized this new element was chemically related to chlorine and fluorine.

Iodine is widely, though very unevenly, distributed in nature. The main supply, however, is in the sea and in deposits recently derived from the sea, as in the guano beds of Chili and the rock salt of Stassfurt, Germany. McGlendon has estimated the total iodine in the sea at 60,000,000,000 metric tons, and each liter of sea water as containing 0.05 mg. All plants and animals living in the sea so far as is known contain iodine. Certain plants, like kelp, and animals, like tunicates, contain sufficiently large amounts of iodine and exist in such quantities that they could be important sources of iodine commercially. Iodine is present in many rock formations and ores, particularly of silver, copper and mercury. Soils and soil waters contain small but highly variable amounts. This fact was first emphasized by Chatin, 1852, who pointed out that goiter was more common in those regions where the soil and water were very low in iodine. In general the soils of coastal plains are highest in iodine and those of glacial origin are lowest. The air and rain water in the vicinity of the sea also may contain traces. Commercially the major source of iodine is at present from the nitrate deposits of Chili and the rock salt at Stassfurt. Prior to the development of these sources, seaweed or kelp was the only source commercially. In sea animals below the vertebrates iodine appears to be diffusely scattered throughout the tissues, both as inorganic and organic compounds. However, very little is known of the exact distribution and form of storage in the plant and in invertebrate animal tissues. In sponge and coral, iodine is localized in the axial skeleton as iodo-gorgonic acid (di-iodotyrosine) and this fact has been suggested as

indicating that iodine was useful in the metabolism of these animals.

Rôle of Iodine in Plants and Lower Animals.—Nothing is known in regard to the rôle of iodine in the nutrition of plants and lower animals. In land plants, like edible vegetables and cereals, iodine frequently exists in traces. This, however, varies with the iodine content of the soil in which the plants were grown, although there is some evidence that even in the same soil certain plants (for example, beets) have more iodine than others. There is no evidence at present that iodine is a necessary food constituent of plants and the lower forms of animal life, although on general biological grounds it is probable that it is a useful, if not necessary, constituent, even though a special tissue devoted to its storage and elaboration has not been recognized. In the absence of such a specialized tissue it is a very difficult problem to investigate, and up to the present no extensive investigations have been undertaken in this field.

Iodine and the Thyroid Gland.—On the other hand in vertebrate animals beginning with the Cyclostomes (Lampreys) a special tissue has been developed in which iodine is stored and elaborated into a hormone essential to the well-being of the animal. This tissue is called the thyroid gland and the normal presence of relatively large amounts of iodine in it was first recognized by Baumann in 1895. The localization and concentration of iodine in a single tissue of higher animals has made it possible and relatively easy to investigate many phases of the rôle of iodine in vertebrate physiology and pathology. The rôle of iodine in health and disease in all the higher vertebrates, including man, therefore, centers

about the thyroid gland and the remaining pages will be devoted to a summary of some of this knowledge.

Anatomy of the Thyroid.—While the location and gross morphology of the gland vary somewhat in different classes of vertebrates, the origin, mode of development and microscopic appearance are essentially identical in all animals from Cyclostomes to man. In man the normal thyroid gland weighs between 20 and 25 grams and does not exceed 0.35 gram per kg. It is slightly larger per unit of body weight in women and relatively much larger in infants, being 1-700 to 1-1000 for infants and 1-2000 to 1-2500 for adults. It is roughly U-shaped and composed of two lateral lobes 5-6 cm. in length by 2 cm. in width, connected across the midline in front of the trachea by an isthmus. The isthmus varies in width from 1 to 2 cm. and in thickness from 6 to 8 mm. The lobes are rather firmly attached to the larynx laterally and the isthmus to the trachea. The gland, therefore, moves with the trachea. The only portion of the gland which is readily palpable is the isthmus. This may be felt to ride under the finger held against the trachea in the suprasternal notch while the individual executes the movement of swallowing. Palpation of the isthmus is therefore of considerable medical importance in that it is the most certain means at our disposal of determining clinically the presence or absence of slight enlargements of the gland.

Physiology.—Our knowledge of the function of the thyroid began in 1874 when Sir William Gull showed that the gland underwent extreme atrophy in cases of myxedema (Gull's disease). The Reverdin brothers (1882) and T. Kocher (1883) independently reported the accidental occurrence of a symptom complex similar

to myxedema following the surgical removal of the thyroid for goiter in man. These observations attracted immediate attention and gave rise to a great deal of experimental work on animals. Most of the experimental work done prior to 1891 was of little value because the parathyroid glands (usually four tiny structures closely adherent to the thyroid) were not known and the symptoms of parathyroidectomy (tetany) were mistaken for those of thyroidectomy. Since 1891 many species of animals have been subjected to thyroidectomy in which modern surgical procedures were used and in which the parathyroid gland factor was eliminated. It was found that removal of the thyroid in many adult laboratory animals, like the rabbit, sheep and the goat, led to very little change in the clinical behavior of the animal beyond a dryness and thickening of the skin, thinning of the hair, a gain in weight and a lowering of body temperature; whereas thyroidectomy done in very young animals caused, in addition, gross manifestations of stunted physical, mental and sexual development.

Following the discovery in 1895 by Magnus-Levy that metabolism (heat production) was markedly decreased in cases of myxedema, heat production measurements have been carried out on all the ordinary laboratory animals after thyroidectomy and a similar marked lowering of metabolism has been observed in them. These studies established the identity of experimentally produced myxedema and spontaneous myxedema. Metabolism may be lowered as much as 40 per cent below the normal and usually begins within five to seven days after removal of the thyroid. The lowering of heat production still remains the most characteristic manifesta-

tion of the loss of thyroid function. In 1891 Murray and MacKenzie cured a case of myxedema by administering an extract of sheep's thyroid. This was the first and is still the only instance where the oral administration of a gland completely replaced and compensated for the normal intact organ. Again in 1897 Magnus-Levy showed that the beneficial effect of the administration of thyroid gland was in raising the level of heat production, and up to the present this remains the most characteristic pharmacological action and therapeutic effect of thyroid medication. In recent years (since 1915) the methods for measuring heat production have been enormously elaborated and exploited in the diagnosis and control of treatment of thyroid diseases.

Discovery of Iodine in the Thyroid.—In 1895 Baumann discovered that iodine was a normal constituent of the thyroid and that no other tissue in the body contained iodine in amounts remotely comparable. This discovery has proved to be the greatest advance yet made in unraveling the physiology and pathology of the thyroid. It at once aroused an enormous interest which resulted within ten years in showing that iodine was the determining element in the specific secretion of the thyroid which caused its most characteristic effect, namely, increased metabolism (heat production), and its absence caused the decreased heat production. Baumann showed that the iodine was held in firm organic combination and isolated a substance which he named *iodothyrin*. Oswald showed that the iodine-containing compound was bound with the globulin or colloid material of the gland. Other workers showed that feeding substances rich in iodine greatly increased the iodine store in the gland and that feeding diets rich in

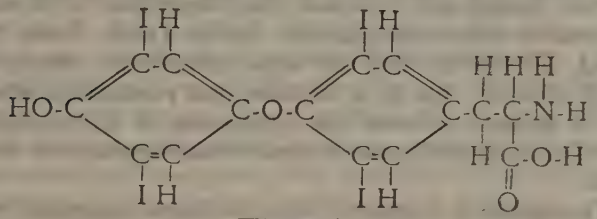
proteins and fat decreased the iodine store. It was also shown that the iodine store was lowest in goitrous thyroids and highest in normal thyroids. The maximum amount of iodine that can be stored in the normal human thyroid following its administration is about 25 mg. or between 5 and 6 mg. per gram of dry gland. The least amount is seen in goiters and may be so low as to be undetectable, while the average iodine store for normal glands is around 2 mg. per gm. of dry gland. These figures also have been found to be true for the thyroids of dogs, cats, sheep, pigs, oxen and other mammals.

Isolation and Synthesis of Thyroxine.—Attempts to isolate this specific iodine-containing substance were made by a great number of biochemists, including Oswald, Hutchinson, Frankel, Drexel, Notkin, Reinbach, Roos and many others, but all of them, including Baumann, failed. In 1916 E. C. Kendall of the Mayo Foundation isolated this stable iodine-containing hormone in crystalline form and named it *thyroxine*. According to Kendall it crystallizes in rosettes and sheaves of needles, melts around 250° C., is insoluble in acid but soluble in strong alkali, contains about 65 per cent of iodine and has an empirical formula, $C_{11}H_{10}O_3NI_3$. From numerous analyses, Kendall and Osterberg in 1919 concluded that this substance was an indol derivative of the following structure:

$$C_6H_4I_3.NH.CO.C.CH_2.CH_2.COOH$$

The inherent chemical improbability of such a formula was universally recognized, but owing to a lack of sufficient thyroxine for analysis, it was not until C. R. Harington of London in 1926 announced the perfec-

tion of a simple method for the more complete extraction of thyroxine that sufficient amounts for study were obtained. Harington's analyses gave thyroxine the formula $C_{15}H_{11}O_4NI_4$. Structurally Harington has shown thyroxine to be beta-tetra-iodo-(3, 5, 3^1, 5^1)-4-(4^1-hydroxy-phenoxy)-phenyl-alpha-amino-propionic acid, with the following structure:



Thyroxine

With this knowledge of the chemical structure of thyroxine, Harington has recently succeeded (1927) in preparing it by synthesis in the laboratory. Lyon has proved (1927) that the artificial product has the physiological effect of thyroxine itself. It may be expected that in a short time chemists will be able to manufacture it synthetically as has been done in the case of the active principle of the suprarenal gland (epinephrine). While the natural drug as contained in dried thyroid keeps well and is effective in the treatment of disease the production of thyroxine artificially would, in addition to being another triumph of synthetic chemistry, enable physicians to control the dosage and obtain the desired physiological effect with greater certainty and precision. Thyroxine is elaborated relatively slowly by the thyroid, although the living thyroid cells have an extraor-

dinary capacity for taking iodine out of the circulating blood. Thus the iodine store of the thyroid may be increased several hundred per cent within a few seconds following the injection of 50 mg. of potassium iodide into the femoral vein. This newly acquired iodine is at first inactive physiologically, but begins to show activity in about twenty-four hours. This fact shows that there is no fixed ratio between the physiologically active and inactive iodine and indicates that thyroxine is slowly elaborated from the inactive iodine taken from the blood. Thyroxine has the same physiologic effects as whole thyroid, that is, it increases heat production when fed to animals, relieves the symptoms of thyroidectomized animals and causes a rapid metamorphosis of tadpoles.

To sum up in a sentence what we know regarding the normal function of the thyroid gland, one may say that the thyroid gland, through its iodine-containing hormone (thyroxine), provides a means for maintaining a higher rate of metabolism than would otherwise obtain and also provides a means for varying the rate of metabolism to meet changing physiologic needs.

DISEASES OF THE THYROID

On the basis of our present knowledge of the function of the thyroid the diseases associated with disturbances of its function may be divided into two broad groups as follows:

I. Thyroid insufficiencies: Simple goiter (endemic, sporadic); infantile (Cretinism) and adult (Gull's disease) myxedema.

II. Graves' disease.

Whether Graves' disease (exophthalmic goiter) should

be grouped with the primary thyroid diseases or not is doubtful. The thyroid changes in this disease appear to be secondary to some obscure derangement of the visceral nervous system and are probably of a compensatory nature. If this view should ultimately prevail the thyroid changes found in Graves' disease would be similar to those found in the thyroid insufficiencies, and all the evidence at present available supports this view, particularly the evidence regarding the storage of iodine, the relation of iodine to the morphological changes in the gland and the effect of iodine administration.

Simple Goiter.—Simple goiter includes those enlargements of the thyroid gland in man and animals which were formerly designated endemic, epidemic, sporadic, physiologic, colloid, cystic, and by numerous other names. It may occur in any land or fresh-water animal with the ductless thyroid. Animals living in the sea are free from the disease. On the sea-coasts generally it is rare and the cases seen are usually in women. It occurs in all races, in all climates and at all inhabitable altitudes. In temperate zones goiter develops more frequently in the late winter and early spring months. While goiter may occur anywhere, even in mid-ocean, one of the most striking characteristics is the increased incidence in certain more or less defined regions of the world, the so-called districts of *endemic* goiter. The most notable of these districts are the Himalaya Mountain region of South Central Asia, the Alps region in Europe, the Andes plateau in South America and, in North America, the region about the Great Lakes and the St. Lawrence River and the Cascade Mountain region of Oregon, Washington and British Columbia. Less important foci occur throughout the Appalachian Mountain region, the Rocky Mountain

states and states in the Central Basin. While most of the world's goiter districts occur in mountainous regions, there are numerous exceptions. Of greater importance is their occurrence for the most part on soils deposited from the melting ice of the last glacial epoch.

The sudden occurrence of large numbers of cases of goiter, so-called epidemics, have for the most part been in military garrisons, in institutions and in schools, in districts where the ordinary incidence of goiter is high.

Acute outbreaks of simple goiter have occurred in dairy herds, on poultry farms and in fish hatcheries, both in districts where the ordinary incidence of goiter was high and where it was low.

Goiter More Frequent in Girls.—Beginning about the age of puberty, girls are affected more often than boys. In districts of severest endemic goiter this difference in incidence due to sex is masked because all of the inhabitants may be affected. In non-goitrous regions where only sporadic cases occur these are usually seen in women. Averaging these extremes, one may say that in general simple goiter is two or three times more common in females. The periods in life when simple goiter most frequently develops are (1) during fetal life, (2) during pregnancy and lactation, (3) during puberty and (4) during the menopause, i.e., during those periods of life when growth, differentiation and energy transformations are greatest.

Cause of Goiter. Iodine Deficiency.—The cause of goiter has interested medical men from the earliest times. St. Lager in 1867 tabulated over forty theories concerning its causation. Most of these theories must now be classed as folk lore, as, for example, that it was due to drinking snow or glacier water, to carrying heavy loads

on the head or to breathing bad air. Poverty, unhygienic living conditions, unbalanced diets, certainly are indirect contributing causes. A great variety of organic and inorganic substances, both of known and unknown chemical nature, occurring in the air, soil and water, have been suspected as causal factors. Among these may be mentioned salts of calcium and magnesium, sulphides, fluorides, silica and miasms from decomposing vegetable matter. These substances are not now believed to play any direct rôle. The conception that goiter was caused by living germs has been widely held and during the last fifty years many types of organisms—bacteria, fungi and protozoa—have been described. However, no proof has been offered suggesting that goiter is either infectious or contagious. It is well known that poisons of bacterial and other origins may cause thyroid enlargements. Thus thyroid enlargements are often seen in early pulmonary tuberculosis, in active syphilis and in acute fevers, like pneumonia, scarlet fever and diphtheria. Water has been associated as the carrier of the goiter-producing agent by all peoples from the remotest times. The Chinese held such views some fifteen centuries B.C. and the inhabitants of Central Africa and the American Indians were as convinced that water caused goiter as were the Greeks and the Romans. A great deal of experimental work has been done with water and in the light of our present knowledge the evidence obtained can be interpreted rather as indicating a lack of some substance (iodine) necessary for the prevention of goiter than the presence of some substance capable of producing it. In 1911 Lenhart and the writer demonstrated that brook trout with goiter regularly recovered even though living in the most polluted water when the goiter-producing diet of hog's

liver was withheld, although all fish living in the same water and fed with hog's liver quickly developed goiter.

As a result of the numerous studies on the relation of iodine to the thyroid gland our present views regarding the cause of goiter assume that it is a compensatory or work hypertrophy of the thyroid depending upon a relative or an absolute deficiency of iodine. This deficiency of iodine may be due to:

1. Factors which bring about an abnormally low intake of iodine.

2. Factors which interfere with the absorption or utilization of an otherwise adequate intake.

3. Factors which increase the needs of the body for the iodine-containing hormone.

Group 1. Factors which bring about an abnormally low intake: This would explain endemic goiter. The idea that goiter is due to a lack of iodine is not new. As early as 1830 such views were expressed. The work of Chatin, published in 1852, deserves special mention. He showed that there was a lack of iodine in the soil and water in districts of endemic goiter. His results were attacked and unfortunately so discredited on the grounds of faulty and inadequate methods that further study of this important work was blocked for forty-five years. During the last ten years very extensive analyses of foods, soils, and water for iodine have been made and all of them confirmed Chatin's claims of a marked reduction of the iodine in soils and waters of goitrous districts. It has also been shown experimentally that goiter may be produced in animals by depriving them of their normal intake of iodine from food and water.

Group 2. We have no definite information at present of factors which cause an interference with the

absorption and utilization of an otherwise adequate amount of iodine. It is conceivable that intestinal bacteria or the lack of some vitamin or hormonal factor could divert part of the normal iodine intake or prevent its utilization.

Group 3. There are many factors which temporarily increase the needs of the organism for the iodine-containing hormone, that is, factors which create a relative insufficiency. Among the more important of these may be mentioned (a) diet, as shown by the production of goiter in brook trout on a diet of pig's liver or the production of goiter in pigeons on an excessive diet of lard or other iodine-free digestible fat; (b) infections, as in pulmonary tuberculosis, syphilis, tonsilitis and focal infections; (c) pregnancy; (d) puberty; and (e) Graves' disease.

The first group depends upon a definite, simply defined and easily understood factor, an actual deficiency of iodine, whereas the third group, which depends on a relative or temporary deficiency, is more difficult to define, less understood and by far the most important, since no living animal with the ductless thyroid is ever free from the possible influence of one or another of the factors enumerated. These factors of diet, pregnancy, puberty, infections, etc., are active in goitrous as well as in non-goitrous districts and when combined with a real iodine starvation as occurs in endemic goiter districts the effects are exaggerated. It is important to distinguish between groups 1 and 3 when planning or evaluating treatment. Emphasis on the iodine deficiency has been so prominent of late that we are liable to overlook more important elements in the etiology and

therefore treatment, particularly in the so-called sporadic cases.

IODINE IN THE PREVENTION AND TREATMENT OF GOITER

Early History.—Iodine in one form or another has been used unknowingly from the earliest times in the treatment of goiter. The beneficial effects on goiter of substances now known to contain iodine is said to have been known to the Chinese fifteen centuries B.C. Of these iodine-containing substances, sponge ash is the best known, and was extensively used at the time of Hippocrates. In addition to its use by the Greeks as an important remedy in the treatment of diseases in general, Dioscorides specifically mentions the use of sponge ash in the treatment of goiter. Galen and Pliny also refer to its use. Humboldt in 1824 described the occurrence of endemic goiter in the United States of Colombia, and refers to the interesting fact that these people knew one salt deposit was more beneficial in the treatment of goiter than another. A young French physician, Roulin, came to Colombia after graduating from the University of Paris, where he had learned of Coindet's work on iodine in the treatment of goiter. He became interested in this subject, and had analyses of these salts made for iodine by Boussingault and found that the deposit containing the most iodine was the one which the natives had found most useful.

Another very interesting reference to the use of iodine unknowingly in the treatment and prevention of goiter is given in Mrs. Lucy Crawford's "History of the White Mountains." After referring to the frequency of swelling of the thyroid gland in Coos County, New

Hampshire, at the close of the seventeenth century, she relates that her grandfather brought sea-salt, a bushel at a time, eighty miles over the mountains on his back. She states that it was generally believed by the early settlers that the swelling of the thyroid was due to the almost exclusive use of meat (deer, moose and other game) which the nature of the country necessitated, together with the lack of salt. They noted that children were more susceptible than adults and recommended that they be given larger quantities of salt, particularly if it was impossible for them to live near the sea a part of each year. This account clearly establishes the fact that long before the discovery of iodine our early settlers knew the value of sea-salt (for this was the only salt available) in the treatment and prevention of goiter. They also were aware of the fact that a diet consisting largely of meats was a potent cause of thyroid enlargement.

Iodine was first knowingly used in the treatment of goiter by the Geneva physician Coindet in 1820—nine years after its discovery. Within a year after its introduction as a remedy against goiter by Coindet, injurious effects were noted in certain classes of cases. These injurious effects were vividly described by Gairdner, who noted that "peculiar great and persevering anxiety, depression of spirits, emaciation, diarrhea, tremor, and nervous excitement simulating chorea" were produced in certain individuals. This symptom-complex was designated "Iodin Basedow's" by Kocher, some seventy-five years later. These early observations on the untoward effects of iodine started a controversy which has now continued for more than a century—whether iodine should be used in the treatment of any form of goiter. There

have always been supporters on each side of this controversy. Up to about 1890 the balance of medical opinion seemed to favor its use in small doses. Then due to the influence, particularly, of surgeons under the leadership of Kocher, and to the overuse of desiccated thyroid introduced in 1891, the pendulum began to shift the other way. Since 1910, however, there has been a gradual return to the older views. This return to the use of iodine has been in great measure due to the rational application of the great mass of physiological facts acquired by and since Baumann's discovery in 1895 of the normal presence of iodine in the thyroid. The gist of all this recent research as applied to modern therapy may be summed up in one sentence—that the thyroid requires an exceedingly small amount of iodine for the carrying out of its function, which so far as we now know consists in the elaboration of an iodine-containing hormone first suggested by the discovery of Baumann and isolated in crystalline form by Kendall in 1916. Undoubtedly the use of massive doses of iodine was the major factor in bringing iodine into ill repute in the earlier empiric treatment of goiter. And if one may draw a conclusion from the recent literature and from my own experience this is still the major cause of untoward effects. All the facts at present known support the view that minimum amounts of iodine are required for the normal physiological needs. Only three of these facts need be mentioned in illustration: (1) That 25 milligrams of iodine represent about the maximum storage capacity of the normal human thyroid. (2) As shown by Marine and Lenhart, 1 milligram of iodine given at weekly intervals by mouth will prevent thyroid enlargement in dogs living under conditions

which would otherwise produce thyroid enlargement. (3) As shown by Boothby and Sandiford, 1 milligram of thyroxine may exert its accelerating effect on metabolism in man for as long as seventy days. With this general background in mind, what would seem the most rational plan of applying these and kindred physiological facts in the treatment and prevention of goiter?

Prevention of Goiter.—The search for means of prevention is not new. This was the main purpose of the Goiter Commissions appointed by the Sardinian Government in 1845, by the Austrian Government in 1860, by the French Government in 1863, by the Swiss Government in 1908 and more recently by the Italian Government. The installation of new water supplies was recommended by some of these Commissions and in certain instances this is said to have decreased and in others to have increased the incidence of goiter. The various suggestions that have been made for the control of goiter have been derived from the prevailing theories as to its cause.

The idea of preventing goiter by the administration of small amounts of iodine also depends on the view that goiter is due to an iodine deficiency. This view has been derived from the great volume of data relative to the physiology, pathology and chemistry of the thyroid, all of which may be summarized in the statement: "If the iodine store in the thyroid is constantly maintained above 0.1 per cent dried weight, no enlargement of the thyroid gland occurs."

The first occasion when iodine was intentionally used for the prevention of goiter on a large scale was in 1909-1910, when Marine and Lenhart demonstrated that this substance added to the water supply in a con-

centration not greater than 1 milligram per liter arrested and prevented the development of goiter in brook trout. It was later shown that changing the diet from pig's liver to hashed whole sea-fish (which contains traces of iodine) was equally efficacious. Since then the administration of iodine in some form or manner has been successfully applied in the prevention of goiter in sheep, cattle, pigs and poultry.

Attempts to apply these methods of prevention in human goiter on a large scale were not successful until 1917, when Dr. O. P. Kimball and myself began experiments with the girls attending the public schools of the city of Akron. The method consisted of giving 2 grams of sodium iodide in 0.2 gram doses, distributed over a period of two weeks and repeated each autumn and spring. The experiment was carried out for two and one-half years and the results were as follows: Of 2190 girls taking 2 grams of sodium iodide twice yearly only 5 developed thyroid enlargement, while of 2305 girls not taking the iodide, 445 developed thyroid enlargement. Numerous papers have since appeared both in America and Europe further confirming and extending the use of iodine in the prevention of goiter.

Simple goiter may be easily prevented in man by the administration of 1 milligram, and probably less, of iodine daily. Iodine in any form and administered in any manner is effective. This fact introduces difficulties and advantages—difficulties regarding the selection of the best form and manner of administration, and advantages in that the desired result may be accomplished with certainty in a great variety of ways. The amount of iodine necessary and the ideal plan of administration for such purposes have not yet been worked out. The

most effective means, and to my mind the least objectionable, would be the use of a salt containing 1 milligram of sodium or potassium iodide per each 10 grams of salt, that is, 0.01 per cent. Such a plan would provide universal prophylaxis at a nominal expense. Where small units of population are to be protected, as for example school populations, iodostarin or starch-iodine tablets containing 5 to 10 milligrams administered once a week may be used. Such a plan could also be readily applied by physicians in the prevention of thyroid enlargement during pregnancy and lactation. Iodine may be introduced periodically into the drinking water following the plan first used by Marine and Lenhart in 1910 in preventing goiter in brook trout. For this purpose iodine in a concentration of one part per million for a period of two weeks each autumn and spring would seem ample. In individual cases in private practice syrup of hydriodic acid is a very convenient and easily obtained form for administration. One or two ounces twice each year is ample. Desiccated thyroid also completely protects the thyroid against enlargement, but it is too dangerous a drug to recommend for this purpose.

Treatment of Goiter.—Regarding the use of iodine in the treatment of thyroid enlargements we cannot be so dogmatic as in the case of prevention. When the treatment can be instituted in the early stages of development of the thyroid enlargements of endemic goiter districts, the methods and the amounts of iodine recommended for prevention bring about a cure. In our Akron experiment where 2 grams of sodium iodide were administered over a period of two weeks twice yearly for prophylactic purposes 773 thyroids out of a total of

1182 with enlargement showed reduction. Similar results have been reported from Switzerland by Klinger and others and from Italy by Pighini. In sporadic goiter the cause of the relative or temporary thyroid insufficiency (infection, diet, etc.) must be sought for and corrected if iodine therapy is to be of permanent value. In the long-standing cases no plan of medical treatment can with certainty accomplish more than relieve any existing functional insufficiency. In these cases the functional insufficiency is usually already relieved or outgrown and the patient seeks the physician's advice largely for the correction of the cervical deformity. Occasionally striking curative results are observed even in long-standing cases. In such cases the thyroids are anatomically in the hyperplastic, simple or colloid form, free from hemorrhages, cyst formation, calcification or adenomata, so characteristic of long-standing human goiter. Whether a curative effect is produced or not, and certainly if the individual expects to seek surgical intervention in the event of failure of medical treatment, iodine should be given. The best plan of medical treatment in my opinion requires the administration of standard U. S. P. desiccated thyroid in 0.1 to 0.2 gram doses daily for a period of two weeks, and if there has been no change in pulse rate, loss of body weight or other evidence of injury, the same treatment may be repeated. Then after an interval of one to two weeks some form of iodine, as for example syrup of hydriodic acid in 2 to 4 cc. doses daily, should be given for a period of two or three weeks. This combined treatment with desiccated thyroid and iodine may be repeated three times during the year, and for the maximum reduction at least a year is required. Desiccated thyroid unquestion-

ably causes a greater reduction in the volume of the thyroid and more rapidly than does iodine. The administration of large amounts of iodine suddenly often causes the thyroid in about seven days to become very firm to the touch, painful and clinically even larger than before its administration (the so-called iodine thyroiditis of the literature). This is due to the rapid accumulation of colloid in the alveoli which iodine induces.

The injurious effects of the excessive use of iodine or desiccated thyroid in the treatment of goiter are real and often serious. Coindet in 1821 first noted these effects in certain types of goiter and Gairdner as above mentioned in 1822 observed and described all the essential features as we know them today. This effect of iodine is most commonly seen in women with long-standing goiter during or after the menopause, which is in some unknown way a factor in causing the increased susceptibility. Because of this element of serious danger in the indiscriminate use of iodine, treatment should always be under the guidance of a medical adviser who can follow the effects of the treatment and modify it as needed. Self-administration is not to be thought of.

Exophthalmic Goiter.—In sharp contrast to simple goiter we know almost nothing concerning the etiology of Graves' disease and until this is known a rational therapy cannot be expected. The prevailing opinion now favors the view that Graves' disease is not primarily a thyroid disease, but is in some way dependent upon a deranged function of the visceral nervous system. This view is only a return to that held by the older internists and neurologists, including Charcot, Trousseau,

Buschan, Gowers, prior to the introduction of the Gauthier-Moebius hypothesis in 1886. My own view is that underlying the typical clinical picture of Graves' disease there is a long-standing constitutional anomaly which may be acquired or inherited (congenital), the acquired form coming on usually in association with or after the menopause, the thyroid hyperactivity being secondary and a result of a powerful stimulation either through the sympathetic nervous system or through the blood stream or both. To determine the origin of this stimulation of the thyroid, as well as that of other tissues, particularly the lymphoid (thymus and lymph glands) is the major problem in connection with this disease. After a careful study of the literature, together with observations and experiments on a large series of our own cases, Marine and Lenhart came to the conclusion in 1910 that the thyroid reaction in Graves' disease was in no way different from that seen in other clinical associations. Oswald in 1902 and A. Kocher in 1912 also fully described the effect of iodine administration in Graves' disease.

We are here concerned more particularly with the use of iodine in the treatment of this disease than with experimental data. Its beneficial effects are limited while its injurious effects are serious. The value of iodine is limited to relieving any real or relative insufficiency of the thyroid. This iodine undoubtedly does, but unless and until the cause of the thyroid stimulation which produces the relative or temporary insufficiency can be relieved, no lasting benefit occurs merely from supplying the means with which the thyroid can manufacture more thyroxine more easily or more quickly. As above mentioned, Coindet in 1821 noted injurious effects

from large doses of iodine in certain types of cases, and Gairdner in 1822 definitely described the symptoms of Graves' disease in connection with his observations on the injurious effects of iodine in certain cases of goiter at least thirteen years before Graves recognized the syndrome as a distinct disease. From the earliest literature on exophthalmic goiter, more than one hundred years ago, just as today, definite benefit has been reported from the use of iodine. On the other hand highly injurious effects have been noted. A great increase in the injurious effects has been noted since the introduction of desiccated thyroid into goiter therapy in 1891. These effects became so serious that many of the ablest students, under the leadership of Theodore Kocher, opposed the use of iodine in any form in this disease, indeed in any form of goiter. Beginning about 1910 the pendulum began to swing back again to the view of the earlier observers, namely, that iodine could be used without danger if administered in doses somewhat approximating what might be called physiological (1 milligram daily). Why some patients undergo remarkable improvement and others do not is not understood.

Summary.—The greatest value of iodine in thyroid disease will always be in prevention. Its value in treatment is limited and conditioned. Simple goiter should be treated with desiccated thyroid combined with iodine. This will relieve any functional insufficiency but as regards the deformity, much depends on the duration of the enlargement and the presence of complications like hemorrhage, cysts, calcification and adenomata. The beneficial effects of iodine in the treatment of Graves' disease are limited, while its injurious effects are serious. Its benefits are limited to relieving real or relative func-

tional insufficiency of the thyroid. This iodine undoubtedly does, but until the cause of the thyroid stimulation in this disease can be relieved, no lasting benefit occurs merely from supplying the means with which the thyroid can manufacture more thyroxine more quickly.

Chapter VI—Article 7

INSULIN TO THE RESCUE OF THE DIABETIC

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Before going to the University of Toronto, Dr. Macleod was professor of physiology at the Western Reserve Medical School in Cleveland for fifteen years (1903-18). In 1923 Professor Macleod and Dr. F. G. Banting received jointly the Nobel prize for the discovery of insulin. Dr. Banting promptly shared the award with Dr. Best and Professor Macleod shared his with Dr. Collip. The discovery of insulin by this group of scientific men represents one of the greatest triumphs of cooperative research in the history of medicine and chemistry. Insulin is not only a great blessing to mankind in itself, but the effect of the discovery in stimulating further intensive cooperation in research cannot be measured. Aside from the Nobel prize mentioned above, Dr. Macleod has been elected to honorary membership in many American, Canadian and European scientific societies and academies.

Diabetes is a disease due to a disorganization of the chemical (metabolic) processes by which the animal body utilizes the foodstuffs for the production of energy. Its first objective symptom is an increase in the percentage of sugar in the blood (hyperglycemia), followed later by the appearance in the urine of sugar (glycosuria) and subsequently of acetone bodies (acetone, acetoacetic acid and β-oxybutyric acid). Left to itself, the disease is often fatal; but, thanks to the efforts of science, it can now be so successfully controlled that fatal issues are very rare and there is every probability that the afflicted person will complete his allotted span of life. Insufficiency of an internal secretion called insulin is responsible for the disorganization of the chemical processes. The pancreas, which is also a very

active digestive gland, is the source of insulin, so that it is not surprising that post-mortem changes can be detected in the pancreas of patients who have died of the disease. The principle of treatment depends on restoring the missing insulin to the blood. This can be done, in mild cases, by restricting the sugar-producing foodstuffs in the diet, so that less insulin is required, and in severer ones, by administering insulin prepared by chemical extraction of the pancreas of the lower animals. Before considering how this is done let us briefly review the long series of researches which finally culminated in revealing these facts and in making insulin readily available to all who may need it; for, as in every other branch of science, the discovery of insulin was the outcome of painstaking researches extending over many years, each new result being a step towards the ultimate goal.

Early Work.—The first step towards the discovery of insulin was really taken in 1683 when Conrad von Brunner observed that removal of the pancreas in a dog was followed by peculiar symptoms which, from his excellent description of them, we now know to have been those of diabetes. Brunner did not realize that he had made a great discovery, his interest being rather to show, by the survival of the animals, that the secretion of pancreatic juice into the intestine was not an essential feature of digestion. It was not until more than a century later (1788) that Cowley, an English physician, suggested a relationship between the pancreas and diabetes. In 1845 this was confirmed by the French physician Bouchardat. As the evidence for the relationship became so strong, experimental investigations began to be undertaken to see whether destruction or removal

of the pancreas in laboratory animals would make them diabetic. No success attended these endeavors until 1889, when two German investigators, Minkowski and von Mering, achieved it, by taking very great care to see that the gland was completely extirpated. They, and others who repeated their work, showed that if even a small remnant of the gland were left in the body the diabetic state might not supervene. The suggestion was made that the pancreas, besides the important digestive function which by this time, contrary to Brunner, it was known to possess, must also produce some internal secretion with the function of controlling the metabolism of carbohydrates in the animal body. Stimulated by these results, investigators of various types, anatomists, physicians and chemists, began seeking for the particular structural element in the pancreas which might be the source of the internal secretion, and attempts were made to prepare extracts of the gland, which when administered to diabetic patients would replace the normal internal secretion and so cure the disease. Some twenty years before von Mering and Minkowski announced their great discovery, an anatomist, Langerhans, had described in the pancreas collections of cells which differed in their microscopic structure from those known to be responsible for the secretion of the digestive juices, and these groups of cells have since come to be known as the Islets of Langerhans. The question arose as to whether these might be the source of the supposed sugar-consuming internal secretion and a number of extremely painstaking and careful researches by the anatomists Laguesse and Diamare lent strong support to an affirmative answer. They were able to show, not only that the islet cells have a highly characteristic structure, but also that

the islets have no ducts and belong therefore to the category of ductless glands, like the thyroids or adrenal glands. Meanwhile pathologists also found that these same islet cells were the ones that became most prominently affected in patients who had died of diabetes. The evidence that the islets secrete into the blood what may be called an anti-diabetic hormone became so strong that Sir Sharpey Schafer suggested in 1916 that this hypothetical secretion, or hormone, should be called "Insulin."

It must be noted, however, that the only evidence supporting these views was as yet purely circumstantial in nature. Although many attempted by chemical methods to prepare extracts of the pancreas that would contain insulin and would therefore relieve the symptoms of diabetes, the results were entirely unconvincing. Perhaps the most encouraging of these early attempts was that of Rennie and Fraser who used as raw material, not the pancreas of mammals, but the peculiar glands, called principal islets, found in certain fishes, which had been shown by anatomical study to be composed of the same cells as those of the islets of Langerhans, but at the same time to be more or less free of pancreatic-secreting cells. Unfortunately, however, they administered their extracts, or even in some cases the raw glands themselves, by mouth instead of by subcutaneous injection, and it is altogether probable that the negative results which they obtained were due to the fact that the strong digestive juices of the stomach destroyed the insulin which recent research has shown such glands to contain in abundance. In one patient, however, a boy, the extracts were administered subcutaneously and in this case the published results do

show a certain improvement in the diabetic symptoms.

Nearing the Goal.—It came to be realized among certain of those working on the problem that the chief difficulty standing in the way of success was due to destruction of the insulin by the strong digestive ferments derived from the main secreting cells of the pancreas, and attention was directed to circumventing this destructive influence. The immediate problem, therefore, was to find some means by which the digestive cells of the pancreas might be eliminated without destruction of those of the islets. It was found that this could be accomplished more or less satisfactorily by tying the ducts through which the pancreatic juice is poured into the intestine. Fortified with this knowledge, E. L. Scott in 1912 attempted to make extracts of the pancreas, the duct of which had been ligated some time previously, but unfortunately he did not obtain sufficient atrophy of the gland. Scott, as well as other observers, particularly Zuelzer (1907), tried another method to circumvent the action of the digestive ferments, namely, by extracting the pancreas with alcohol, in which the digestive ferments are inactive. Zuelzer's results were distinctly encouraging. He prepared acid alcoholic extracts of calf pancreas, then removed the alcohol at low temperature and dissolved the residue in saline solution. He thus obtained an extract which he injected intravenously into diabetic patients after showing that it was effective when given to diabetic animals. The general symptoms of the disease were definitely relieved and the excretion of ketone bodies and of sugar in the urine became much less, or disappeared entirely. Unfortunately, certain of these patients developed obscure symptoms of an alarming nature which made

further trial of the extracts inadvisable. In the light of our present-day knowledge it is possible that these baffling symptoms may have been due to overdosage with insulin, thus causing the blood sugar to fall to the level at which convulsions supervene. Had Zuelzer only paid closer attention to the effects of the extracts on laboratory animals before trying them on diabetic patients it is altogether likely that insulin would have been available in 1908. Numerous other investigators, such as Murlin, Clarke, et al., meanwhile obtained results which, although unconvincing, nevertheless had in them sufficient that was positive to keep alive the hope that a method would at last be found by which the elusive hormone would some day be effectively extracted. Murlin deserves great credit for the persistent and painstaking efforts which came very near to being successful in 1916.

The Successful Issue.—In 1921 Dr. F. G. Banting undertook in my laboratory to make another attempt to extract insulin from the pancreas. Having read that ligation of the pancreatic ducts is followed by atrophy of the digestive secretory cells, leaving the islet cells more or less intact, he started, with the collaboration of C. H. Best, to reinvestigate the problem by using extracts of the atrophied gland. The effects of the extracts were studied on diabetic dogs; in this part of the investigation a distinct advantage over previous investigators was gained by having available, as a result of biochemical research, accurate micro-chemical methods for estimation of the percentage of sugar in the blood, an increase in which, it will be recalled, is the cardinal symptom of diabetes. It was decided to consider the behavior of the

blood sugar as the indicator as to whether or not the extracts had any anti-diabetic influence.

All possible duct connections between the pancreas and intestine were therefore ligated in several dogs and after a month or so had elapsed, so that the gland might have time to atrophy, simple saline extracts were made from the atrophied pancreas. These extracts were injected intravenously into other dogs in which diabetes had been induced, with the result that an immediate and definite lowering of the blood sugar occurred. Similarly prepared extracts from other tissues did not have this effect. These encouraging results were frequently repeated to make sure that there could be no mistake. The supply of atrophied gland being naturally very limited, it was next decided to see whether active extracts could also be obtained from the undegenerated gland. Knowing that the digestive ferments do not become active until about the time of birth of an animal, Banting and Best prepared extracts from embryonic pancreas. These also were successful in lowering the blood sugar and the result was a stepping-stone to using adult (cattle) pancreas, as obtained from the slaughter house. In this case the destructive action of the digestive ferments was circumvented by the use of alcohol and the extract was further purified by removing the fat present in it by means of toluene. A saline solution of the residue was found to contain insulin, and it was now considered justifiable to see whether its administration to diabetic patients might be of advantage.

After some extract was passed through a Berkefeldt filter, so as to remove bacteria, it was injected subcutaneously into a diabetic patient, with definitely beneficial results. It was found at the same time, however,

that before being further tried the extracts would require to be considerably purified, so as to remove substances from them which were causing irritation at the site of injection. At this time J. B. Collip was invited to join in the investigation, his special problem being to purify the extracts. Finding that the blood sugar in normal rabbits is lowered by the extracts, Collip was able, in a comparatively short period of time, to prepare a tolerably non-irritating extract. His method depended on fractional precipitation of the crude alcoholic extracts with different strengths of alcohol. The results now made it possible to prepare a sufficient amount of non-irritative extract with which to treat other cases of diabetes, and it did not take long to show that all the symptoms of this disease are relieved by insulin.

The Problem of Large Scale Production of the Hormone.—The quantities of extract were at this time extremely limited, since the work was only being done on a laboratory scale. It became necessary to secure larger amounts, and with the collaboration of the Connaught Laboratories attention was now turned to this problem, but with results which were at first extremely unsatisfactory and disappointing. Indeed, for some months (in 1922), although every stage of the laboratory process was apparently being duplicated step by step on the larger scale, it seemed impossible to obtain anything like the expected yield of insulin. It almost looked as if we had lost the secret for preparing insulin. Some unknown factor had evidently crept in, leading to the destruction of the hormone during its large scale extraction. It did not seem to be merely the long time taken in the process, for sometimes the yields were reasonably high; it did not seem to be changes in the reaction of the extracts,

for this was controlled by the use of buffers; nor could it be shown to be due to any impurity in the alcohol or other reagents used in the process. It was several months before the difficulties were gradually overcome, great assistance being here obtained by collaboration with the research laboratories of Eli Lilly and Company. It has since transpired that a chief cause for the difficulty depended on inadequate control of the degree of acidity at certain stages in the extractive process, for it was shown by Shaffer, Doisy and Samogyi that the isoelectric point at which maximum precipitation of insulin occurs is pH 4.6 (a concentration of 0.000025 for the hydrogen ion H^+), around which degree of acidity it is certain that many of the extractions in the first attempts on a large scale were conducted. Not only did it become extremely important at all stages of extraction and purification to bear this fact of isoelectric precipitation in mind, but the precipitation itself soon came to be used as the basis for the further purification of insulin. All the insulin sold on this continent is now purified by isoelectric precipitation,* the insulin in the crude alcoholic extract being also concentrated by preliminary treatment with ammonium sulphate. It may be of interest to some if we outline briefly the present-day method in this country.

The pancreas (belly sweetbread) is put through a meat chopper and collected in an equal volume of ordinary alcohol (95 per cent) containing 25 cc. concentrated sulphuric acid for each kilogram of pancreas used. After being stirred for some time, the mixture is filtered,

* *Editor's Note.*—Isoelectric precipitation is a contribution from the field of physical chemistry: it is invaluable in the purification of other important principles, such as antitoxins, etc.

or centrifuged, and the alcohol is removed from the extract in a vacuum still. The watery residue is then mixed with ammonium sulphate (40 grams for each 100 c.c. of solution) whereby a precipitate containing most of the insulin separates out. The precipitate is collected and dissolved in water and reprecipitated with ammonium sulphate. This second precipitate is shaken with water containing sufficient ammonia to bring the pH value to between 6 and 8. By centrifuging, a clear watery solution is obtained from which insulin is precipitated, by adding acetic acid to bring pH to about 5. After standing some time at a low temperature the precipitated insulin is collected on a filter, redissolved in water containing sufficient hydrochloric acid to bring it into solution and reprecipitated by adjusting pH to between 5 and 6. Insulin can also be purified by precipitation with picric acid and the picrate further purified by treatment with alcoholic hydrochloric acid, when it forms a colorless compound called insulin hydrochloride. This is known as Dudley's method and is the one used by the British manufacturers.

It really does not matter which of these two methods is employed. The end product in both cases is a practically colorless precipitate which can be dried in a vacuum desiccator and is of fairly uniform strength. A solution of this precipitate in dilute hydrochloric acid of an acidity greater than pH 4.6 is the insulin of commerce. It is sold in solutions put up in sterile bottles and the solution is of such a strength that 1 cc. contains either 10, 20 or 40 units (see p. 309).

The Chemically Pure Hormone.—Although repeated reprecipitation by either the isoelectric or the hydrochloric acid method finally results in products of con-

stant potency these are, nevertheless, far from being pure in the chemical sense. From 13 to 17 per cent nitrogen and considerable quantities of sulphur are present, but there is no phosphorus. The isoelectric point at which the purest of these preparations, dissolved in pure water, is precipitated lies between pH 4.3 and 5.7. Insulin remains in solution outside these limits unless sufficient strong acid is added to lower pH below 2. In the presence of small concentrations of inorganic salts the isoelectric point shifts towards the acid side and a large number of the salts of the heavier metals also precipitate insulin. Insulin is readily adsorbed, especially in acid solution, by such materials as kaolin, charcoal, benzoic acid and Lloyd's reagent, and this fact is of interest because it can be taken advantage of for purification of the product. Insulin does not dialyze through parchment or collodion sacs, although some does so by the method of electro-dialysis. It is by no means a labile substance, since a solution withstands a boiling temperature, provided the reaction be maintained faintly acid. The instability of insulin in the presence of alkali is of interest, since it may be related to the splitting off of free sulphur. The solutions give certain of the color reactions for protein.

J. J. Abel and Geiling have more recently succeeded in further purifying insulin, by the use of phenol and pyridin, and they have obtained it in the form of minute colorless crystals which are several times stronger in their anti-diabetic effects than the purest dry preparations previously obtained. The crystalline product contains sulphur in an unstable combination. Abel believes that the amount of this sulphur is correlated with the activity of the insulin.

Efforts of Synthetic Chemistry.—Synthetic substances having insulin-like properties (causing a lowering of blood sugar) have also been prepared by Frank, Nothmann and Wagner. These are compounds with guanidine (amino-amyl-guanidine and amino-butyric-guanidine) and one of them (named synthelin), the chemical composition of which is not revealed, is sufficiently potent so that from 3 to 4 mg. per kg. body weight can cause the blood sugar in rabbits to become lowered to the point at which convulsions occur. These substances produce their insulin-like effects slowly, and they are effective when given by mouth. This is undoubtedly an advantage, since insulin must be given subcutaneously. It is to be remembered, however, that useful though these synthetic preparations may be in the treatment of mild and moderate cases of diabetes, they are too slow in action to be of use in severe cases, especially when coma is threatened. It has also been claimed that a material prepared from the myrtle and other related plants has a beneficial action in diabetes, but this is certainly very feeble compared with that of insulin and it is doubtful whether such preparations will prove to be of much advantage.

The most interesting possibility to look forward to is that Abel and Geiling, by analysis of the crystalline insulin which they have obtained, will succeed in revealing its exact chemical structure and thereby in devising methods for its synthetic preparation. It must be evident that the method of preparation of insulin described above and the careful determination of its potency, which will be presently discussed, must make insulin somewhat expensive as thus obtained from natural resources in spite of the very generous attitude of

its discoverers. A successful synthesis of the hormone might well open the way to a very much cheaper, as well as purer, product. The first step toward the synthesis was taken by the preparation of insulin; the second step by the isolation of the pure crystalline principle by Abel and Geiling. Determination of the structure will be the third step necessary before an attempt to prepare the product by artificial means can be made.

Standardizing Insulin.—A most important step in the manufacture of insulin for use on patients is determination of its anti-diabetic strength, for otherwise the physician would not know how much to inject. This biological or physiological assay is based on the power which insulin has of lowering the percentage of sugar in the blood of normal rabbits. It is an easy matter, by pricking the ear, in this animal to obtain at frequent intervals sufficient samples of blood for micro-analysis of the sugar percentage. Immediately after the injection of the insulin the blood sugar, which normally stands at 0.115 per cent, falls rapidly and in an hour or so, depending on whether the animal was recently fed, it declines to somewhere about 0.045 per cent. When it reaches this level peculiar symptoms manifest themselves, namely, violent convulsive seizures which last a minute or so and recur at frequent intervals, followed by the gradual development of a comatose condition and a fall in body temperature. These symptoms are evidently related to the fall in blood sugar (hypoglycemia), since they can be instantly relieved by restoring sugar to the blood by injecting a few cubic centimeters of a solution of glucose (10 per cent) subcutaneously or intravenously. On account of the striking frequency with which these symptoms first appear at a blood sugar per-

centage of 0.045, it was decided, for purposes of the physiological assay of insulin, to consider as one unit that amount which would lower the blood sugar to 0.045 per cent in a rabbit of average size (2 kg.) within an hour or so. In applying this general principle in practice it was found advantageous to modify the above definition somewhat, and today a unit is defined as one-third of that amount of insulin which is capable of lowering the blood sugar to an average of 0.045 per cent over a period of five hours following its injection. But it really does not matter now what the actual definition of the unit may be, since the principle has been adopted of determining the strength of each new batch by comparison with a standard preparation of dry insulin hydrochloride. This comparative method of assay was recommended by the Committee on the Standardization of Toxins, Drugs, etc., of the League of Nations, and a large quantity of highly purified insulin hydrochloride has been set aside, in the custody of the National Medical Research Council in London, to serve as a standard with which each laboratory engaged in the assay of insulin may compare its own standard as necessary. The practice at present is that each of these laboratories carries out its assay by comparison with its own standard preparation of insulin, which, in turn, is compared, from time to time, with the international standard kept under the auspices of the League of Nations. Uniformity in the strength of insulin over the whole civilized world has thus been provided for. This is of great advantage, since otherwise the diabetic patient on moving from one country to another would run serious risks from inaccurate dosage of this potent remedy. If the insulin were too powerful the blood sugar would be too

greatly lowered, causing symptoms of the same nature as those observed in rabbits, and if it were too feeble the diabetic symptoms would return. Of course, if symptoms of hypoglycemia do supervene they can be promptly relieved by taking sugar, orange juice or candies being usually employed. If the symptoms are so severe that the patient cannot swallow, glucose solution must be injected into a vein.

Insulin in the Treatment of Diabetes.—With regard to the exact dose of insulin required in diabetes each case must be a law unto itself, and it requires careful study by the physician to determine how much insulin should be given in relationship to the diet. The object aimed at must always be to hold the blood sugar at about the normal level. The urine will then remain free of sugar and ketone bodies, and the diseased Islets of Langerhans of the pancreas, being relieved of the necessity of secreting insulin, will have a chance to recover. For it is the guiding principle of therapeutics to rest the disordered function which is the cause of the disease, in order that the natural recuperative powers of the tissues may have a chance to develop. Insulin can never cure; it can only replace the insulin which is not being secreted from the diseased Islets of Langerhans. But in so doing, it allows these structures to pass from a state in which, though worn out by disease, they were striving to produce enough insulin for the body's needs to one in which they can, as it were, devote all their remaining vital powers towards restoration of their damaged functions.

In the comparatively few years (five) during which insulin has been available it is impossible to say to what extent actual recovery of the Islets may have occurred in

cases treated with it. But it is certain that even the most acute and desperate cases of diabetes can be brought back to a state that is practically normal and, not infrequently, that the dose of insulin can be considerably reduced. This is particularly the case in those cases of diabetes in adults in which for some reason or another (dietetic indiscretion, infectious disease, etc.) there has been an acute exacerbation of the symptoms and a threatening of coma. Proper treatment with insulin almost invariably saves these cases. Although the dosage may have had to be considerable at first, it can soon be reduced and sometimes even brought to a vanishing point, if the diet continues to be controlled so as to maintain the blood sugar at its normal level.

One other use of insulin may be alluded to, namely, when surgical operations have to be performed on diabetic patients. These used to be very risky indeed, but nowadays practically all danger is removed by preliminary treatment with insulin.

Insulin Saves the Lives of Children.—In children, in which diabetes usually runs a very acute and fatal course, rapidly getting more and more severe in spite of all dietetic control, insulin really works miracles. In a very few weeks after starting its use the child, previously brought by emaciation virtually to skin and bones, comes to look perfectly healthy and it continues thereafter to grow and to put on weight at the normal rate, provided of course that insulin be daily injected and the diet be properly controlled. I know of several children who, at the time insulin was discovered, were obviously very near to death from diabetes in spite of the most competent care and treatment, and who are now alive and in apparent good health, performing their daily

duties and entering into the physical and intellectual pursuits of their fellows.

Such striking results, which are in the experience of every physician, make statistical evidence redundant. In so far as this goes in the short time during which insulin has been available it amply confirms the experience of the individual physician.

Insulin an Aid to Progress in Science.—Quite apart from its great therapeutic value insulin is also a most valuable scientific tool. It places in the hand of physiologists and biochemists an agent by which he can cause profound changes to be brought about in the metabolism of normal and diabetic animals. Investigation of the nature of these changes is already giving us important information with regard to the manner in which the various proximate principles of food play their respective rôles in the production of the energy of life. In this connection it may be of interest to refer to the effect of insulin treatment in the case of two dogs from each of which the pancreas was entirely removed over three and one half years ago. Both animals are at present in perfect condition, pets in the laboratory. Insulin has to be injected subcutaneously twice daily, and since this is done immediately preceding feeding time, the animals leap and bark with joy and they salivate profusely whenever their attendant appears with the hypodermic syringe. There is one thing in the treatment of these animals that differs from that of diabetic patients, namely, they have to be given some pancreatic gland along with their food, in order to supply the digestive ferments which they cannot secrete into the intestine. If this part of their treatment be omitted, food is not adequately or properly digested and death occurs after

a few months, associated usually with profound toxic changes in the liver due probably to absorption of improperly digested food. A diabetic patient does not require to take pancreas with his food because, however severe the case may be, there still remains intact sufficient of his own pancreas to provide the necessary digestive ferments.

Chapter VI—Article 8

THE INTERNAL SECRETION OF THE PARATHYROID GLANDS

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Dr. Collip has been called (1927) to the Professorship of Biochemistry of McGill University in Montreal. In 1923, when Professor J. J. R. Macleod and Dr. F. G. Banting received jointly the Nobel prize for the discovery of insulin, Professor Macleod shared his award with Dr. Collip and Dr. Banting shared his with Dr. Best.

The parathyroid glands are minute structures occurring as a rule in two pairs and anatomically closely associated with the thyroid lobes. They have been recognized in all classes of vertebrates except fishes. In addition to the two pairs of glands in each animal, there are found also, on occasion, accessory glandules. The parathyroid gland of the human subject is a bean-shaped structure 3 to 15 mm. long and 2 to 3 mm. broad. It is yellowish brown to brown red in color. The parathyroid glands develop as thickenings of the endoderm of the third and fourth branchial clefts.

The parathyroid glands were first described by Sandström (1880), whose contribution was, however, overlooked for some years.

Parathyroid Glands and Tetany.—Complete removal of the parathyroid glands results in the manifestation of a condition known as tetany, which, if untreated, results, as a rule, in death within a few days of the operative removal of the glands. Experimental tetany due to extirpation of the parathyroid glands was ob-

served by early workers on the thyroid gland and was at that time erroneously attributed to removal of the thyroid gland. The reason for this error is due to the fact that in removing the thyroid gland these observers unknowingly removed the parathyroid glands also—structures which at that time had not yet been described. Gley (1891) showed quite conclusively that tetany and acute death following thyroidectomy were not related to loss of the thyroid gland, but to removal of the parathyroid glands, since removal of the latter glands alone produced the same result.

Tetany Relieved by Calcium Therapy.—Since the work of Gley the parathyroid glands have come to be recognized as vital structures. Their physiology, however, remained quite obscure until 1909, when MacCallum and Voegtlin demonstrated a greatly reduced calcium content of the blood following removal of the parathyroid glands. They showed also that the injection of a soluble calcium salt such as calcium chloride will relieve an attack of tetany and restore the animal to normal for a period. Later Luckhardt and Goldberg (1923) showed that completely parathyroidectomized dogs can be kept free from symptoms by vigorous calcium therapy. Salveson (1923) obtained similar results and related the whole symptomatology of parathyroid deficiency to a lowered calcium content of the blood. The calcium content of the blood is, he held, normally controlled by the parathyroid glands.

It is beyond the scope of this article to discuss in any detail various other aspects—of which there are many—of parathyroid physiology. The parathyroid glands are related most intimately to the metabolism of calcium.

They may play an important part in other phases of metabolism, but of this little is yet known.*

Extracts of the Hormone.—The proof that the parathyroid glands accomplish their function in the intact body by the production of an internal secretion is of comparatively recent date. Such proof consists in the extraction of the hormone from the glands of oxen removed at the time of slaughtering, and the use of such an active extract to afford complete substitution therapy by injection into completely parathyroidectomized animals.

Parathyroid extracts have been prepared and used by many from time to time, but consistent results were not obtained until as a result of the recent work of Hanson, Berman, Collip and associates, a standardized extract is now available.

Moussu (1898) claimed to have arrested post-operative tetany in dogs by subcutaneous and intravenous injections of extracts prepared from the parathyroid glands of the horse. Vassale (1905) introduced a parathyroid gland extract which he asserted had a favorable effect upon tetany attacks in the human subject. Berkeley and Beebe (1909) prepared a parathyroid gland extract by the use of acetic acid. They claimed that a nucleoprotein which they separated had curative properties when administered to parathyroidectomized dogs.

Hanson (1923-24) published a series of papers in which he describes the preparation and use in both experimental animals and man of a weak hydrochloric acid extract of parathyroid glands of oxen made by

* For a full discussion of this question, see Volume I, "Endocrinology and Metabolism," edited by Barker. D. Appleton & Co., New York, 1922.

treating the fresh glands with boiling dilute acid. Berman (1924) claimed to have obtained crystalline material from an extract of parathyroid glands made by the use of acid alcohol, from which extract lipins and proteins had first been removed; a solution of this material in Ringer's solution and injected into the circulation raised definitely the calcium content of the blood.

Collip (1924) prepared an extract by first treating the fresh or acetone-preserved glands of oxen with an equal volume of five per cent hydrochloric acid for one hour in a boiling water bath. Later the further purification of the extract and a method of standardizing the potency of the hormone contained therein were described by Collip and Clark. Collip proved that his extracts were active by the consistent relief or prevention of tetany in parathyroidectomized dogs. Complete replacement therapy was demonstrated under the most rigid conditions. It was shown also that the blood-serum calcium was elevated in the treated animals. A parathyroid extract which had the power to relieve or prevent tetany in experimental animals had also the power to raise the level of blood-serum calcium in a normal dog. The former action, namely, the therapeutic effect, could be accurately predetermined by the later action, namely, the calcium-mobilizing effect in the normal dog.

In the preparation of an extract of the active principle of the parathyroid gland for use in medicine, a long process of purification is necessary. Repeated solution and repurification at the isoelectric point at the end of the process contributed materially to the purity of the product. The extract contains other material with the hormone, but all deleterious impurities have been removed.

Standardization.—For clinical use, the purified extract is sterilized by passing the solution adjusted to a pH of about 4 through a Berkefeldt filter, which removes bacteria. It is standardized in terms of dog units, the unit being the amount which will produce an average increase of 5 mg. in the blood-serum calcium of normal 20-kg. dogs in fifteen to sixteen hours. One dog unit is taken as the equivalent of 100 clinical units and a suitable preparation for clinical use is so adjusted that 1 cc. contains 20 clinical units. The potency test should be made on a number of dogs (at least ten) and the results averaged. A convenient way is to inject the animals in the late afternoon and take readings the following morning.

The Hormone.—Very little is known as yet about the chemistry of the active principle. The best extracts most probably contain much inert material. As in the case of insulin, no doubt much further progress will be made in the future toward the isolation of the active principle as a chemical individual. Proteolytic enzymes such as pepsin and trypsin render the best preparations completely inactive. This suggests that the active principle is a protein derivative and it may be proved finally to be a polypeptide.

The potent extract is characterized by its property of raising the level of the blood-serum calcium. This effect, which follows either subcutaneous or intravenous injection, is observed to occur in a very orderly manner. The serum-calcium curve rises gradually for a number of hours following the injection of the extract, then after plateauing for a short period gradually falls to the normal level. The height and duration of the curve

vary with varying dosage. This result is illustrated graphically in Chart I.

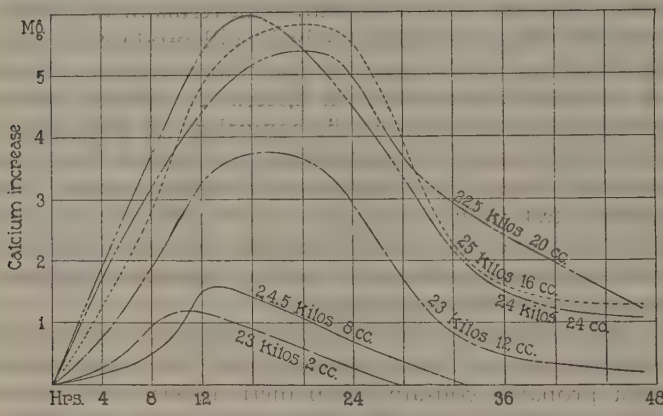

CHART I. Constructed curves showing effect of increasing dosage of parathyroid hormone (single injections) upon blood-serum calcium values of normal dogs.

Single injections of the hormone have not as a rule any ill effect upon normal dogs unless the dosage is excessive. If the serum calcium rises more than 50 per cent of the normal value vomiting may occur, but this in itself is not serious. If massive doses are administered in single injections the blood-serum calcium may rise to 20 mg. or more per 100 cc. and the animal may die on the second or third day. If the condition of profound hypercalcæmia is not maintained for an undue length of time, uneventful recovery may occur.

Repeated injections of even small doses of the extract at short intervals result invariably in death in dogs so

treated. The calcium curve in such cases breaks as a rule a few hours before death. These animals manifest a typical train of symptoms which are undoubtedly associated with the state of profound hypercalcæmia induced by the injections. The physical properties of the blood are altered as well as the blood chemistry.

There is a peculiar species difference in the response to injections of the extract. Thus, dogs seem very susceptible to it while rabbits are relatively unaffected by doses which produce definite effects on dogs. Parathyroidectomy, however, is followed in the rabbit as in the dog by a fall in blood calcium and such an animal may be kept free of tetany by small doses of the extract.

The human subject responds to injections of the parathyroid hormone by an elevation in the level of blood-serum calcium.

It is not the purpose of this paper to discuss the clinical side of this problem. Suffice it to say that a potent extract of the parathyroid glands has been used successfully in the treatment of parathyroid tetany both acute and chronic. As calcium plays such an important rôle in physiological economy, it is possible that the hormone may ultimately be found of service in other conditions where it may be found helpful to influence calcium metabolism.

The problem as to how a probably complex organic substance, such as the parathyroid hormone, functions in the intact body to regulate the calcium content of the blood is still unsolved and in itself presents a most fascinating line of inquiry.

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CHAPTER VII

The Policing of Civic Life in the Laboratory

Article 1

SAFEGUARDING THE WATER WE DRINK

JOHN F. NORTON, Ph.D.

Associate Professor, Department of Hygiene and Bacteriology, University of Chicago

Dr. Norton was instructor and assistant professor of Sanitary Chemistry at the Massachusetts Institute of Technology (1911-17) before he was called to a chair in Bacteriology at the University of Chicago (1917-to date). He is co-author (with A. G. Woodman) of a book on *Air, Water and Food* and editor of *Standard Methods of Water Analysis* (1925), and of *Standard Methods of Milk Analysis* (1927) of the American Public Health Association.

THE TESTING OF WATERS

ALTHOUGH the ancients appreciated the fact that bad drinking water and sickness were often closely related, they had no conception of the reason for this relationship. Time, money and engineering genius were not infrequently expended in order to supply communities with pure water, but no means were available for testing the potability of the supply obtained. It is only during the last half-century that methods have been devised for this purpose.

Chemical Methods.—The earliest tests to determine the purity of drinking waters were solely chemical in character and were based upon the formerly prevalent idea that organic matter was a harmful ingredient. The so-called "oxygen-consumed" test was devised as a rapid method for estimating the amount of organic matter in water. In this test the sample of water was boiled with acidified potassium permanganate and the amount of this reagent reduced by the organic matter in the water was determined. The result was expressed in terms of oxygen. The method is still used as part of the routine chemical analysis in some laboratories, although the sanitary significance of the test is small. With the realization that only certain kinds of organic matter in water were significant, other analytical procedures were instituted.

Typhoid and paratyphoid fevers, cholera and dysentery are the important water-borne diseases. The germs of these diseases enter water supplies with sewage. The presence of sewage in drinking water is therefore indicative of the possible presence of harmful bacteria. Sanitary analysis, chemical or bacterial, consists of tests for the presence or absence of sewage by utilization of substances characteristic of sewage but found in pure waters only in almost infinitesimal amounts. The most important of these chemical compounds are those containing nitrogen—organic nitrogen compounds, ammonia, nitrites and nitrates. The finding of abnormal quantities of nitrogen in its various forms together with an excess of chlorides (usually present in the form of common salt) indicates to the sanitary water analyst that

sewage in some state is reaching the water supply.* The particular form in which the nitrogen is present, together with the amount, is a fairly accurate index of the danger and extent of pollution. This interpretation of the results of chemical analysis is based upon a knowledge of the nitrogen cycle.

When proteins putrefy, simpler organic nitrogen compounds are first produced and finally urea and ammonium compounds are formed. All these products of putrefaction are present in fresh sewage and if found in a drinking water in excessive quantities are sufficient evidence on which to condemn the water. Instead of determining the total organic nitrogen present, the water analyst tests for what is called the "albuminoid ammonia." This is defined as the amount of ammonia set free by the action of boiling alkaline potassium permanganate on the organic nitrogenous substances. The advantage of "albuminoid ammonia" over the total organic nitrogen is twofold—simplification of the analytical procedure and the possibility of distinguishing between organic matter of animal and of vegetable origin. "Ammonia" as determined in water analysis includes not only that present in ammonium compounds, but also that set free from organic compounds, such as urea, by boiling in the presence of a small amount of alkali. Fresh sewage pollution as indicated by the results of these analytical methods is dangerous pollution.

The next stage of the nitrogen cycle is concerned with the oxidation of ammonium compounds to nitrites under the influence of certain nitrifying bacteria. The presence of abnormal quantities of nitrite in a drinking

* See Woodman and Norton, "Air, Water and Food," chap. 5. John Wiley & Sons, 1914.

water together with an excessive amount of sodium chloride also indicates pollution, but the sewage either has not reached the water in a fresh condition or else has remained in the water for some time. Good drinking waters are free from nitrites, containing less than 1/100 part of nitrogen in the form of nitrite per million parts of water. While the indicated sewage pollution is not as fresh as when free and albuminoid ammonias are present, it is, however, sufficiently recent to render the water unsafe for consumption.

Nitrates are formed by the oxidation of nitrites—again, under natural conditions, through the activity of bacteria. Their presence in excessive amounts, together with an abnormal quantity of sodium chloride, indicates what is known as "past" pollution. The time required for oxidation to nitrate, or the conditions under which this takes place in the soil, results in the elimination, at least to a large extent, of intestinal bacteria. Waters which show evidence of past pollution are usually not immediately harmful, but are potentially dangerous, as it is impossible to predict when fresh sewage may enter the water supply. Nitrates may come from other sources than sewage—for instance, from a field or garden treated with commercial fertilizer. Such possibilities must be considered in interpreting the results of the chemical analysis.

Sodium chloride has been mentioned in connection with the nitrogen determinations. Excessive amounts of this salt in a drinking water usually enter with human wastes. It is not always easy to determine whether or not excessive quantities are present, since the anticipated normal amount will vary with the source of the water and the region from which it comes. Good surface

waters, except those close to the ocean, contain but small amounts of salt, and waters from the same locality generally give consistent analytical results. Ground waters vary very much in their salt content and it is necessary to know the so-called "normal" for the particular type of water being examined. Deep wells sunk into rock give such varied results that the analysis for chlorides is of relatively little value.

Given a properly collected sample, together with as much information as it is possible to obtain concerning the source of the sample, the chemist can determine with a fair degree of accuracy whether or not a water supply is safe for human consumption. There is a tendency for the public to regard the sanitary chemist as a sort of magician and to think that he ought to be able to give his report on the basis of his laboratory findings alone. In fact, some people go so far as to believe that the less information furnished to the analyst, the more unprejudiced his decision will be. This is quite erroneous, for the interpretation of the results of the chemical analysis of a drinking water is dependent on so many considerations that only with complete information concerning a water can the chemist come to a definite and correct conclusion as to its potability.

Other Chemical Determinations.—Other methods of chemical analysis are sometimes used in connection with water examination. Among these may be mentioned mineral analyses and particularly those methods which determine the hardness of waters. Such tests are necessary in connection with commercial uses of water, as for steam production in boilers or for manufacturing operations, and also in some instances for the proper operation of water purification plants. It has been shown

that the presence of mineral matter in water is not a consideration in the health of the "normal" individual with the exception of the iodide content. Goiter is quite generally conceded to be the result of iodide deficiency in the diet. Several cities in this country are now attempting goiter prevention by occasional dosing of water supplies with sodium iodide. It is too soon to evaluate the efficacy of this measure. With the exception of goiter, however, no definite pathologic condition has been unquestionably traced to the mineral content of a public water supply. There is certainly no statistical indication that inhabitants of hard water regions are less healthy than are persons habitually using soft waters.

Another method of chemical analysis which has been of some value from a sanitary standpoint is the determination of the oxygen dissolved in a water. While the oxygen content cannot be used as an index of the purity of a drinking water, it can be of service in estimating nuisance conditions in a river or harbor. When such bodies of water receive too large amounts of sewage offensive odors and unsightly conditions may result. The putrefactive processes responsible for such nuisances take place under anaerobic conditions—that is, under oxygen tensions less than atmospheric. The percentage of oxygen saturation in a stream, for example, is a rough index of general sanitary conditions. Unfortunately there is no agreement as to the amount of dissolved oxygen which a stream should contain in order to prevent its being a nuisance, but it is probably fair to state that when the oxygen content averages lower than 25 per cent of saturation there is reason to

believe that the body of water is receiving too much putrescible matter.

The dissolved oxygen determination may also be used for another purpose. The primary object of sewage purification is the production of a stable effluent from an easily putrescible influent. In other words, sewage is treated with the object of nuisance prevention rather than to eliminate disease germs. Indeed we have but little satisfactory knowledge of the fate of the typhoid bacillus in the modern sewage purification processes, although we have reason to believe that the conditions during treatment of sewage are not those most favored by these bacteria. If unstable sewage is mixed with water containing oxygen, this gas quickly disappears. If, however, properly stabilized sewage is used in the mixture, the oxygen content changes slowly. The rate of decrease of dissolved oxygen is therefore a measure of the stability of a sewage effluent.

Bacterial Methods.—While chemical methods for testing potable waters were developed before biologic procedures, at the present time it is generally conceded that the latter methods give the more satisfactory results. The principle involved in the interpretation of bacterial methods of water examination is fundamentally the same as that described for the chemical analysis. The bacteriologist does not waste his time in a futile search for typhoid bacilli or cholera vibrios. Like the sanitary chemist, the sanitary biologist uses the presence of sewage in drinking water as an index of the unsuitability of such a water for human consumption. Although a knowledge of the total number of bacteria per cubic centimeter of water may be of value under certain circumstances, it is far preferable to use a more easily

interpreted procedure. While the chemist uses the presence of characteristic sewage chemical compounds as indicative of the presence of waste material in a drinking water. the bacteriologist utilizes a characteristic sewage organism—the colon bacillus. This name refers rather to a group of bacteria than to a single species. The organisms are found in the intestinal tract of man and the higher animals. A species closely related to *Bacterium coli* has been isolated from soils, grasses and grains. However, for most purposes, the group of bacteria known as the coli-aerogenes group may be used as a safe and satisfactory index in sanitary water analysis. These organisms should not be continually present in a good drinking water in more than one out of five 10-cubic-centimeter portions.

A chemist may appear to be somewhat less interested in biologic than in chemical analyses. It is no exaggeration to state that just as the chemist must have a knowledge of physics, the bacteriologist would be helpless without a fundamental chemical training. The sanitary biologist recognizes the colon bacillus in a water not through morphologic studies but by biochemical reactions. The supplying of proper food and of a favorable chemical environment is essential for the growth of an organism. Bacterial nutrition is a relatively unexplored field and our knowledge of the multitude of chemical compounds produced as the result of bacterial metabolism is woefully lacking. Only by active cooperation of the chemist and the bacteriologist—and it is a courageous individual who would claim to be both—will progress be made along these lines. Just as one example, take the various media used to obtain bacterial growth. The most common media contain, as

basic ingredients, meat extracts and peptone. To these may be added a variety of materials, such as carbohydrates, blood or blood serum, bile, etc. The exact chemical composition of the basic ingredients, as well as that of many of the added substances, is unknown. There is no doubt that extracts of meat and peptones vary much in their composition. These variations affect bacterial growth. The many reported discordant results in the examination of milk may be partially accounted for by the varying chemical constituents of the media. For a long time bacteriologists have used so-called synthetic media, but on the whole these media have not proved as satisfactory as those utilizing mixtures of protein split products such as commercial peptones. If the bacteriologist could have at his command media of known composition, a great step in advance would be made. Undoubtedly such media can and will be prepared as the result of cooperative effort on the part of the chemist and the microbiologist.

WATER PURIFICATION

The Filtration of Water.—Water purification by filtration dates in the United States from 1870, when an English-type slow sand filter was built at Poughkeepsie, N. Y. Rapid filters were introduced in the 1880's. In 1925 there were more than 600 filters, largely of the rapid type, in use in this country, with an approximate capacity of 5000 million gallons per day. During the last forty years many improvements have been made in both the slow sand and rapid methods of water filtration.

The slow sand filter depends on the formation, on the surface of a sand bed, of a slimy gelatinous mass

composed of a variety of organisms and their metabolic products. This material acts as a bacterial filter. The slow sand filter is a highly efficient mechanism for removal of bacteria from water. The development of this filter is one of the notable achievements of American sanitation, and credit is due almost solely to the Sewage Experiment Station at Lawrence, Mass. Unfortunately, it is impracticable to treat many American water supplies on these filters, on account of the necessity for the removal of relatively large amounts of suspended matter or color. Color is only partially removed while excessive quantities of suspended matter interfere with economical operation of the filters. From a chemical standpoint these filters are of relatively little interest since they involve no chemical problems except in so far as coagulants have been used—a recent development involving the principles employed in the other method of filtration.

The rapid or mechanical filter is chemical and physical rather than biologic in principle. A brief description of the process is as follows. The water is dosed with some chemical which will form a coagulum with the alkali in the water. Sulphate of aluminum is commonly used for this purpose, although ferrous sulphate is sometimes employed. If the alkali content of the water is insufficient it is increased to the proper amount by the addition of soda ash, lime or calcium carbonate. The precipitated aluminum hydroxide is allowed to coagulate and this precipitate adsorbs colloidal and other suspended matter, including bacteria, from the water. About one-half of the coagulum settles out in suitably constructed basins and the remainder is removed in sand filters. The aluminum hydroxide caught by the sand

also acts to remove suspended matter not picked up in the coagulating basin.

The rapid filter gives a reasonably good bacterial removal and an almost complete removal of color and inorganic suspended matter and it can be operated at a high filtration speed—usually around 125 million gallons of water per day per acre of filter surface. This rate is from thirty to fifty times as high as slow sand filters can be run. The location and housing of rapid filters are thereby made easier and at a less cost for land. Over 580 of this type of filter plant are in use in this country at the present time.

Chemical control of these filters is a necessity. The amount of alum needed must be determined. The alkali content of the water must be known. Both these factors will vary at different times of the year. The amount of alkali, if any, to be added to the water must be ascertained and analyses of all the chemicals used must be made. The presence of dissolved carbon dioxide in the water interferes with proper coagulation. The amount of this must be ascertained and if necessary aeration must be used for its removal.

Rapid filters are fundamentally physico-chemical in their action. Increased knowledge concerning the adsorption processes involved would seem to be necessary for future development of rapid filtration.

The effectiveness of water filtration in safeguarding the public health is best estimated by a study of death rates from typhoid fever before and after the operation of filters. A few instances, among many available examples, will suffice to show the value to the public of this method of disease prevention. In Philadelphia the typhoid fever death rate dropped from 61 per 100,000

population in 1907 to 35 in 1908 with one-half the water supply filtered and to 22 in 1909 with 90 per cent filtration. In Pittsburgh the typhoid fever death rate dropped from 125 in 1907 and 45 in 1908 to 23 in 1909 following the introduction of filtration. The following table gives a few other data.

TABLE I

Effect of Filtration on the Typhoid Fever Death Rate*
(Rate per 100,000 population)

City	Typhoid fever death rate	
	Before filtration	After filtration
Cincinnati	50	12
Columbus	78	11
Albany	74	22
Lawrence	114	25

The Chlorination of Water.—While the introduction of water filtration was a big step toward the protection of the public against water-borne diseases, it was found not to be entirely adequate. Filters are not absolutely reliable. The necessity sometimes arises for operation of filters at higher rates than they are designed for, in order to furnish a sufficient amount of water to a community. Furthermore many water supplies of fairly good quality are in use and it is doubtful if the expenses of installing and operating filters would be justified. Some further form of protection against filter troubles and against the casual, or sometimes small but continuous, pollution of raw drinking water supplies is desirable. This protection can be most satisfactorily obtained by treatment of water with a chemical disinfectant. The practical application of chemical compounds to drinking waters for the purpose of killing bacteria

* *American Journal of Public Health*, 1913, III, p. 1327.

has, so far, been limited to chlorine and its compounds.

Although experiments with hypochlorites appear to have been made in this country and in England as early as 1896-97, the first systematic treatment of a large water supply was carried on under the direction of Sir Alexander Houston in England in 1904-05. Sodium hypochlorite was used in amount sufficient to supply one part of chlorine per million of water. In America, chlorination was begun in 1908. Calcium hypochlorite (chloride of lime) was used as the dosing solution almost exclusively for the following five years. The hypochlorite solution was usually prepared from bleaching powder, and the nuisance involved in handling this material undoubtedly retarded its use. Following the experiments of Major Darnall in 1910, apparatus for the introduction of liquid chlorine into water was developed and became generally available about 1913. Chlorination has been rapidly extended since then, until at the present time more than one-half the population of this country is being supplied with chlorinated water. This corresponds to about four billion gallons of water per day.

Liquid chlorine is easily applied to any public water supply. After the proper dosage has been determined, the chlorinator can be set at the proper point and dosing is automatic. Of all forms of water treatment this is the least expensive and in certain circumstances the most effective. There is general agreement among sanitarians as to the inadvisability of supplying a community with a raw water. Even if the supply comes from an unimpeachable source, there is always the possibility of contamination during transit to the consumer. This is ap-

parently what happened in the last great water-borne typhoid fever epidemic in this country.

The amount of chlorine applied to a water will vary with each supply. Filtered waters generally, but not universally, require smaller doses than raw waters. The amounts actually used vary from 0.15 part of chlorine per million gallons of water to about 0.7 part. Chlorine, even in these quantities, is a perfectly harmless ingredient, but it must be remembered that chlorine rapidly disappears, due to its absorption by organic matter in the water, so that the consumer receives far smaller amounts of chlorine. Complaints are not infrequently heard concerning the disagreeable odor and taste of chlorinated water. Some of these complaints are products of the imagination, but others are real, particularly where raw waters containing even minute amounts of certain trade wastes are treated. It has been fairly well established that chlorinated phenols are the most common cause of objectionable odors and tastes, but there is no evidence that these substances, in the quantities present, are in any way harmful.

There has been a good deal of discussion as to the mechanism of the destructive action of chlorine on bacteria. The nascent oxygen theory is found in most textbooks, but is unsupported by any good evidence. To the bacteriologist it appears that chlorine or hypochlorites act as direct poisons for the bacterial cell. It must also be remembered in water treatment that chlorine acts as rapidly on dead as on living organic matter and that waters high in such substances will require comparatively large doses of chlorine.

The same criteria as used to test the value of filtration may be applied to chlorination. Bacterial analyses indi-

cate the effectiveness of the treatment, but typhoid fever death statistics furnish the most impressive evidence. Table II gives a few representative results.*

TABLE II

Chlorination and Typhoid Fever Death Rates (per 100,000 population)

City	Chlorination begun	Typhoid death rate Before using	After using	Per cent Reduction
Cleveland	Sept. 1911	35.5 (1900-10)	8.2 (1912-16)	77
Erie	Mar. 1911	50.6 (1906-10)	15.0 (1912-14)	70
Jersey City	Sept. 1908	18.7 (1900-07)	8.4 (1909-16)	55
Toronto	Apr. 1911	31.2 (1906-10)	7.8 (1912-16)	75
Kans. City, Mo.	Jan. 1911	42.5 (1900-10)	14.2 (1911-16)	66

Other cities have had similar experiences with chlorination. With an estimated cost of chlorination of one cent per capita per year, the protection afforded is truly remarkable. Although liquid chlorine has proved to be the most satisfactory form for application to municipal water supplies, some cities still use bleaching powder and a few employ electrolytic bleach (sodium hypochlorite).

Attempts have been made to use other chlorine compounds. The organic chloramines are excellent germicides, but their cost is prohibitive. Mixtures of hypochlorite and ammonia have, however, been successfully applied. The advantage of this method appears to be found in the lower dosage of chlorine required for treatment when ammonia is present.

The Elimination of Odors and Tastes.—While odors and tastes in drinking water may be disagreeable, they are invariably harmless to the consumer. This question will, therefore, be dealt with very briefly, since its health

* Race, "Chlorination of Water." John Wiley & Sons, New York, 1918.

significance is secondary. A public water supply may be unsatisfactory to the consumer because of a pronounced color, the presence of suspended matter, an excessive amount of iron or other mineral matter, or objectionable odors or flavors. Such conditions may cause the consumer to abandon the public water supply in favor of some local well or bottled water. At times these other waters are less pure than the discarded municipal supply. It is therefore desirable that the public be supplied, as far as feasible, with an attractive as well as a safe drinking water.

Aside from odors resulting at times from chlorination, disagreeable conditions may be caused by growth of certain organisms, particularly in reservoirs. Trouble may also accrue to the water-works engineer, for excessive growths may result in the clogging of pumps and mains. The organisms involved include both lower plant forms such as diatoms and algae, and also the lower animal organisms—the protozoa. The different species produce their characteristic odors. For example, asterionella gives a geranium odor, volvox a fishy smell, anabaena a vile grassy odor and synura a cucumber taste. Some of these organisms are quite sensitive to chemicals, others are relatively resistant.

Three methods have been used to cope with such growths. Aeration is often a great aid and is sometimes sufficient. Chlorine, sometimes in rather large doses, has been found in some cases to be a good destructive agent. The most common method of control, however, is by the application of copper sulphate. The amount of this chemical required usually varies between one and four pounds per million gallons of water. Continuous dosing is not necessary, since these organisms have a seasonal

occurrence. There is no evidence that the use of copper sulphate in reservoirs is in any way deleterious to the health of the consumer.

Chapter VII—Article 2

SAFEGUARDING THE FOODS WE EAT

FRED O. TONNEY, M.D.

Director of Laboratories and Research, Chicago Department of Health

Dr. Tonney has been associated with the laboratories of the Department of Health of the City of Chicago since 1906, and he has been Director of the Laboratories and Research since 1909, with direct responsibility to safeguard the food supply of the city.

To the technology of chemistry and its sister science, bacteriology, we are principally indebted for the modern methods of safeguarding foods. Every well-organized health department now has its division of food control and manufacturers and growers of foodstuffs everywhere are utilizing the chemical laboratory to improve their product, increase production, develop new and better methods and find new uses for their output.

ADULTERATION OF FOODS

One of the earliest developments in the field of food control was the detection of adulteration. This was made possible by chemical analysis.

Milk.—For years the unscrupulous dairyman had been overworking the pump-handle in an effort to make his product go farther; or perhaps he was in the habit of taking off some of the cream and selling the remainder as whole milk. The food chemist soon put a stop to this. The specific gravity of the milk is changed by adding water or abstracting fat, and solids also are lessened. The lactometer and the butter fat tester told the story in court, and the practice of watering milk became un-

profitable. When the city laboratory of Chicago was established first as a milk-testing laboratory in the year 1894, the percentage of watered samples was found to be 23.3! In 1925 it was 0.3, a negligible amount from a practical standpoint.

Cream and Butter.—Then followed the adulteration of cream with artificial thickeners, such as gelatin and sugar of lime. These again were detected by the chemist by careful analysis and the practice was stopped. Butter came in for adulteration with foreign fats, such as beef suet and coconut oil. Rancid butter was worked over or "renovated," but the chemist promptly devised methods of detecting these frauds.

Tomato Ketchup.—The story of the vicissitudes of tomato ketchup is an interesting chapter. When the market for this product developed to profitable proportions, a few short-sighted manufacturers began to adulterate their output with pumpkin pulp and apple pulp, the waste of the cider press and canning factory. The spurious product had to be colored to make it resemble the genuine, and to this end anilin dyes similar to those used in the textile industries were pressed into service. Some of these dyes proved to be poisonous and their use in foods opened another chapter in the history of food control. The enhanced attractiveness of the artificially colored ketchup made it superior in appearance to that manufactured from pure tomato pulp. Partly decayed pulp of the tomato, as well as its substitutes, was also used in making ketchup, and to prevent rapid spoilage of this inferior product, chemical preservatives came into use. In solving the problem of control of such abuses the food chemist was able to detect readily the artificial colors and preservatives, but could not identify

foreign pulp or distinguish spoiled from fresh ingredients. He therefore called upon his neighbor at the universities and agricultural schools, the plant histologist. The student of plant life brought to light the fact that the minute cells of which the tomato is composed have recognizable differences from those of the pumpkin and apple and that these differences are readily distinguishable under the microscope. Microscopic methods were soon developed for recognizing foreign pulp and also decayed pulp in tomato ketchup and for estimating the amount of such ingredients present with a degree of certainty acceptable to the courts.

As a result of the activities of the food chemist there developed standards of purity for ketchup and other products, a list of permissible non-poisonous colors, exact knowledge of the safe dosage of preservatives such as sodium benzoate, and legal requirements as to truthful labeling, which now not only protect the consumer but also protect the legitimate producer against unfair competition.

Sweets.—There are many other possibilities of food adulteration which our limited space does not permit us to discuss in detail. There is the use of saccharin, an excessively sweet coal-tar derivative having no food value whatever, as a substitute for sugar in candies, soda pop, jellies and canned fruits. There is the story of adulteration of candies with talcum and paraffin, of their tinting with poisonous colors, of starch added to powdered sugar, of ground coffee mixed with chicory root, soya beans, acorns, etc., and of strained honey made from glucose. Even the industrious and well-intentioned honey bee was tempted to adulterate his product by the device of placing bowls of glucose syrup

near his hive. Honeycomb can be quickly filled with spurious sweets by bees working in this manner and the product has all the appearances of genuine honey made from flowers. The polariscope of the chemist's laboratory detects these substitutes, taking advantage of the peculiar properties of various kinds of sugar to rotate the plane of polarized light passing through their solutions.

Vinegar.—The making of artificial vinegar was also for a time a profitable trade practice. The fermentation of apple cider and wines to make vinegar gives rise to an acid called acetic acid which imparts to the product its sour taste. There are also fruit flavors and extractives in naturally fermented vinegar, not easily duplicated in an artificial product. Acetic acid, however, is more cheaply made by destructive distillation of wood, and the acid made in this way is the basis of the artificial vinegars. Such products, however, lack the flavor and consistency of real fruit vinegars and are apt to be too acid. In the past some manufacturers tried the use of the strong mineral acids in the preparation of vinegar. Diluted sulphuric acid and hydrochloric acid were used for a time. These, however, are dangerous substances in food products and the practice of using them was soon stopped. Artificial vinegars must now be labeled to distinguish them from true fruit vinegars.

THE GRADING OF FOODS

The data of food analysis are being used not only to repress harmful trade practices, but also for the very constructive purpose of grading the quality of foods. Certain kinds of foods now have their market value determined by analysis. This applies particularly to milk

and its products, to flour and to sugar wherever these foodstuffs are bought and sold on a large scale.

Milk Products.—The farmer now sells his milk to the creamery on the basis of its butter fat content. Large buyers, such as hotels, restaurants and institutions, contract to purchase milk having from 3 to 4 per cent butter fat. Cream is commonly graded as "market cream" of 18 per cent, "rich cream" of 22 per cent and "whipping cream" of 30 per cent butter fat content. Cheese also is graded and sold on its butter fat content, the richer grades bringing a higher price. Butter is graded by analysis, and purchased on specifications. The official standard for the best grade of butter requires at least 82.5 per cent of butter fat. This allows for about 12 per cent of water and 5.5 per cent of other solids. Tests to exclude oleomargarine and renovated butter are included in the stipulations.

Flour and Breadmaking.—For many years wheat flour has been sold on the basis of chemical analysis. The various grades of "white flour," "second grade flour," "whole wheat flour" and "graham flour" are determined by their content of protein, "gluten," moisture, crude fiber or bran ash and by baking tests. The chemist has also extended his field of usefulness from the analysis of raw materials into the process of breadmaking itself. Bread is no longer made by the old wasteful and haphazard methods. Each step is standardized. Control begins with analysis of the raw products, the flour, the yeast and the salt. The process is followed throughout and the acidity of the dough is adjusted or hastened by the addition of lactic acid. Calcium sulphate is added to speed up the growth of the yeast organisms. The temperature and humidity of the rising chambers

are controlled by automatic machinery and the processes are timed by automatic devices. In fact the modern bakery is in many respects a huge laboratory, which has made an exact science of the baking process.

Sugar.—The sugar industry has seen large advances within the memory of the present generation. The application of the polariscope to the grading of sugars and syrups from the coarsest grades up to the finest highly refined "granulated" product, so pure that it is marketed as 99.5 per cent sucrose, has furnished an exact basis for fixing quality and price.

PRESERVATION OF FOODS

The problem of preservation of foods has occupied the attention of chemists for many years. The use of salt, sugar, saltpeter and spices for this purpose has long been known. A few decades ago attempts to preserve foods by the addition of new chemicals resulted in several serious mistakes. The tendency on the part of food manufacturers was to use chemicals whose effects on prolonged administration by mouth were too little known. Formaldehyde in milk and cheese was one of those which proved to have definite poisonous effects on human beings. Formic acid, boric acid, borax, fluorides, fluorosilicates, sodium benzoate, sodium salicylates and sodium sulphites all came into use while there was still a lack of sufficient knowledge of their possible toxic effects.

The "Poison Squads."—The situation became so grave that the Department of Agriculture of the United States Government was forced to take a hand in the matter. Under the auspices of this Department and in cooperation with several large universities, "poison

squads" were formed for the purpose of studying the effects of chemicals by prolonged administration. Groups of volunteers were fed varying doses of the chemicals proposed as food preservatives. The studies were carried on for several years and were very exhaustive in nature. The findings of these researches are expressed in the federal and state food laws now in force. Most of the chemicals studied are prohibited in any amount whatever in foods. A few are allowed in a dosage determined to be safe provided their presence is stated on the label. The commonest of these are sodium benzoate in quantities of not to exceed one-tenth of one per cent in beverages, and sodium sulphite in amounts not exceeding 350 milligrams per kilogram of foodstuffs.

New Methods of Preserving Foods.—Researches in food preservation have been carried still further. The time-honored practices of smoking and pickling of meats, which are essentially methods of chemical preservation, have come under close scrutiny in the laboratory. The smoke of the hickory bark is now available in liquid form. This "condensed smoke" or creosote is made by distillation of wood. "Wieners" and other sausages, instead of being "smoked" for several days or weeks, are immersed or soaked in the condensed smoke preparation. The chemical principles of this form of preservation are the same by both methods. The time element, however, is greatly shortened by the newer process, and the dosage of the essential chemical creosote is under more accurate control.

Chemists investigating the old process of pickling hams and bacon by means of saltpeter have found that the essential preservative of the process is not the saltpeter itself, but a small amount of another chemical,

sodium or potassium nitrite, which is formed from saltpeter by the action of bacteria. A logical simplification of the process, therefore, was to substitute a small amount of sodium nitrite for the saltpeter. This was done in extensive experiments with very excellent results. It was found that less sodium nitrite could be used than was often formed in hams cured by the original process and that the dosage could be more accurately and safely controlled.

On the basis of these experiments a recent ruling was made by the United States Department of Agriculture, permitting sodium nitrite to be used instead of saltpeter in amounts to yield not more than 200 parts per million in the finished product.

Other Ways of Preserving Foods.—Other processes of food preservation are not being lost sight of in the search for safe and effective chemical preservatives. Cold storage, a development of not so long ago, has grown to an industry of vast proportions. Sterilization by heat, which means killing the germs of decay, by heating the foodstuffs in a closed container which can at once be sealed against the entrance of more germs, is the accepted method of preservation of the canning industries.

A relatively new development in food preservation, an outgrowth of the ancient method of sun drying of fruits and meats, has lately been coming to the front. Vacuum drying, a rapid process of desiccation, is now being applied to milk, vegetables and certain fruits formerly dried by slower and more wasteful methods.

FOOD SPOILAGE AND BACTERIAL CONTAMINATION

Sanitary Control of Foods.—Modern health departments give much attention to enforcement of general

sanitary regulations in food establishments. Corps of inspectors are kept in the field to check on the cleanliness of iceboxes, utensils, floors, counters, etc., to guard against sewage contamination and to insure proper facilities for personal cleanliness of employes. Special attention is given to certain kinds of food products which are consumed in the raw state, such as milk and ice-cream, because disease germs accidentally introduced into such foods are not destroyed by cooking as is the case with most foods.

Municipal milk supplies are subjected to rigorous inspection all along the route from the producer to the consumer. Inspection begins on the dairy farm, where emphasis is given to the cleansing and sterilizing of utensils, to scrupulous cleanliness in milking and handling, to prompt cooling of the product after it is drawn and to keeping it cool during storage and transit.

At the country creameries or collecting points, other control steps are applied. Milk as it arrives from the farms is given the "visible dirt" test, in which dirt is filtered out on a cotton pledget, and the "methylene blue reduction test," in which dirt and contamination have the effect of decolorizing a blue dye in varying time intervals. depending on the quality of the milk. Occasionally bacterial samples are taken and sent to the central laboratory for examination. Producers whose product fails to pass these tests are notified to improve their methods and on a second bad test are given a "shut-off" order by the controlling health agency. Farmers whose product is shut off must appear personally in the Health Department before their product is again accepted.

Reports of contagious disease on the farms and among

employes of milk establishments are required from the country creameries as a precaution against spread of disease through milk. The appearance of contagion on a farm or in a milk plant results in a shut-off unless the infected person is removed at once from the premises.

When the milk reaches the pasteurizing plant in the country or city it is again under the close supervision of the Health Department. The equipment is checked by sanitary engineers, the temperature to which the milk is heated is recorded by automatic devices and the time of exposure to the heat is accurately controlled. Scrupulous cleanliness must be observed throughout the process of pasteurizing and bottling. Bacterial samples are collected and examined in the Health Department laboratories. Again as the milk passes from the plant on its way to the consumer it is subjected to bacterial tests. Final containers are collected in plants, stores and wagons and brought to the laboratory for test. The effectiveness of these methods in preventing milk-borne epidemics is well shown by the experience of the city of Chicago, in which no epidemic in any way traceable to milk has occurred since the year 1916, when the present system of milk control was established.

Inspection of Slaughter Houses.—Inspection of slaughtering houses to detect diseased animals destined for food, and of the packing and canning establishments to insure the quality and safety of the product, are functions of combined federal, state and municipal health agencies.

Examination of Food Handlers.—A recent development in the control of food establishments is the physical and laboratory examination of certain groups of food

handlers for evidences of typhoid fever, dysentery and tuberculosis.

Milk and ice-cream handlers are now routinely examined by health departments of the larger cities and this measure is also, to an extent, applied to employes of restaurants and hotels.

Laboratory Tests of Food.—The testing of foods as they appear on the market is largely the function of the public health laboratory.

Methods of testing canned foods for spoilage have been developed by the chemist. The swollen cans are punctured and the gas analyzed. The inside of the can is examined for corrosion and the contents tested as to odor, appearance, acidity, ammonia content and the presence of poisonous metals.

Fresh meats, fish and fruits are tested for spoilage. Fowl are commonly examined for evidences of "roup" or fowl plague and tuberculosis. In the year 1926 the Chicago Health Department condemned 432,196 pounds of fowl and game for these causes.

Recent epidemics of typhoid fever in several cities of the United States, due to contaminated oysters which were eaten raw, have focused attention on shell fish and their protection against contamination with disease germs. Researches conducted in the Chicago Health Department laboratories and elsewhere have demonstrated that typhoid germs may live as long as sixty days in shell oysters. Bacterial studies of shell fish have resulted in the adoption of a standard laboratory test known as the "colon bacillus score" to judge the sanitary quality of oysters and other shell fish. The colon bacillus is a germ found in sewage, and for this reason its presence

in large amounts in shell fish is accepted as suggestive of dangerous contamination.

The federal and state governments of shell fish producing regions also exercise control over oyster beds, taking steps to protect the beds and shucking plants against sewage contamination and prohibiting the marketing of shell fish from sources known to be polluted.

Liquid eggs, which consist of the poorer grades of edible eggs, broken into metal containers, and preserved by being frozen in the solid state, have a large market for use in making bakery goods, salad dressing and for cooking purposes.

The egg, once its shell is broken, is inherently subject to spoilage and for this reason has been much investigated by food chemists. Numerous tests have been developed which give information as to the quality of eggs used and their state of preservation. These include physical tests such as the odor, appearance and consistency; bacterial tests, of which the most important is counting the number of bacteria; and chemical tests, of which there are many, such as total solids, an index of watering, acidity of the fat, an evidence of rancidity, the ammonia nitrogen, a rough indication of decomposition, and tests for the presence of chemical preservatives.

In the early history of the industry many condemnations of spoiled liquid eggs were made by health authorities as unfit for food. With the improvement in methods of control, however, the product has improved in quality and it is but seldom that condemnation of this foodstuff is found necessary.

Botulism.—Botulism is a very fatal form of food intoxication due to a germ called the *Bacillus botulinus* or the "sausage bacillus." The organism is present in

soils of many localities and hence may readily find its way into vegetables, meats, etc. It was first described in connection with spoiled sausage, which was responsible for an outbreak of fatal poisoning. It has frequently been found in spoiled olives, canned spinach, peas, beans and similar products. The germ, in growing for several weeks or months in food products, elaborates a chemical toxin which is extremely poisonous. It is this toxin which, when eaten with the food, is responsible for the sudden illnesses and deaths which occur.

Laboratory studies have shown that the prevention of outbreaks of botulism lies mainly in the thorough sterilization of foodstuffs when they are canned or packed. This is accomplished by heating the product to a temperature known to destroy all germs, including the *Bacillus botulinus*.

Other Germs Causing Food Poisoning.—Other germs are sometimes responsible for food infections due to canned goods. The principal ones of these are paratyphoid bacilli and the other members of a group of organisms known as salmonella. All of these infections can be prevented by the proper sterilization of food when it is canned.

POISONS IN FOODS

Poisons found in food fall into two general classes. (1) poisons arising from manufacturing processes or growing methods and (2) poisons introduced by accident or design. The presence of small amounts of metallic poisons in food has long engaged the attention of food chemists. Traces of arsenic have been found in candies, in gelatin and in beer due to the use of impure chemicals in the manufacturing processes. Lead and zinc

in canned goods come from drops of the solder used to seal the cans, which fall into the contents and are dissolved.

Lead in "Pops."—Several years ago chemists of the Chicago Health Department discovered that lead in varying amounts was regularly present in "pops" and other carbonated beverages then on the local market. Investigation revealed that the lead came in part from the patent lead stoppers of the bottles, and partly from the carbonating apparatus used to charge the contents of the bottles. This at first seemed surprising, as metallic lead was known to be practically insoluble in pure water. It was shown, however, that the charging process, which introduced carbonic acid gas into the water under pressure, greatly increased the solubility of lead in the water. Large amounts of lead were found in some bottles of "pops" and instances of lead poisoning occurred in children who drank it.

As a result of these findings lead stoppers were barred from the Chicago market by the Health Department and were replaced by stoppers of block tin. Lead pipes and soft solder were prohibited in connection with the charging machines. Other localities followed suit and in a short time lead had disappeared from these products. No further trouble in connection with them has since been experienced.

Arsenate of Lead on Fruits.—A more recent development has been the discovery of poisonous amounts of arsenate of lead on apples and grapes grown in certain districts where insecticides are necessary and the rainfall is insufficient to wash off the residue before the produce is harvested.

A suit was recently brought in Chicago by the United

States Department of Agriculture against a Pacific Coast grower, to prevent the sale of a shipment of apples containing more than 0.01 grain of arsenate of lead per pound of fruit. On the testimony of well known toxicologists and public health officials, the court sustained the contention of the government that the apples were unfit for food. The apples were, therefore, barred from the market unless properly cleaned to remove the poisonous residue.

Poisonous Coloring Agents.—The art of food coloring as it is practiced today adds much to the attractiveness and appetizing quality of prepared foods. Artificial coloring of foods began with the use of a few simple vegetable and animal colors such as annatto, turmeric, cochineal and indigo. The greater durability, effectiveness and economy of the anilin dyes, however, prompted a trial of the latter coloring agents. As in the case of food preservation they were at first used without sufficient preliminary knowledge of their possible poisonous effects. As a result instances of poisoning occurred which tended to establish prejudice against the proper use of perfectly harmless coloring agents, and greatly retarded the legitimate development of this branch of the dye industry.

Anilin dyes containing arsenic and lead as impurities were used for a time. Dyes which were inherently poisonous were also used in a few instances. Even metallic poisons were sometimes introduced into food for tinting purposes, as in the case of pickles colored with copper sulphate.

The food chemist, however, has changed all this. All toxic coloring agents have now been effectively put out of use in food. A list of harmless coloring agents, known

as "allowable colors," has been established and in most cases written into the food laws or regulations. There is no longer any practical danger in consuming foods artificially colored by approved methods.

Poisons Introduced into Foods by Accident or Design.—Control agencies every now and then encounter instances of poisoning due to intentional or accidental introduction of poisonous agents into food.

A recital of a few such instances coming under the observation of the Chicago Health Department will serve to illustrate this phase of food control.

The Case of Jean Crones: On the evening of February 10, 1910, a young man named Jean Crones, evidently suffering from a form of insanity known as paranoia, and believing that he had a grievance against society, put a quantity of arsenic in the soup served at a banquet held in the University Club of Chicago. Two hundred and ninety-six persons, including many prominent Chicagoans, attended the banquet. During the dinner people suddenly began to fall ill. A well known surgeon who was present diagnosed the attacks as "ptomaine poisoning." Vomiting was the most prominent symptom and the fact that it occurred early probably saved the lives of many of the victims. No inkling of the nature of the poison was had until the following morning, when the Health Department investigated the case. Samples of the various foods served were brought to the city laboratory and the whole force of chemists was assigned to the search for poisons. Within one-half hour of the receipt of the samples one of the chemists had discovered arsenic in the soup and soup stock. The news was sent out by telephone and through the daily press, and all persons affected were urged to consult their

physicians at once. Later tests developed that the amount of arsenic present was about 1 1/3 grain in each serving of soup. The copper kettle in which the soup was heated had a heavy coating of arsenic, which had been deposited in accordance with well known chemical principles, and this fact fortunately had served to reduce materially the amount of arsenic served to the guests. The perpetrator of the crime was never caught. He had evidently planned carefully both the act and his escape. In his room were found many chemicals and articles of laboratory apparatus. He had been taking a correspondence course in chemistry and had evidently been awaiting a favorable opportunity to apply his newly found knowledge and satisfy his grudge against society.

Fluorides in Biscuits: In 1924 a housewife living in Chicago mistook a bag of roach powder for flour and made some biscuits of it for her family. As a result four members of the family died and another barely recovered after a serious illness. Samples brought to the Health Department laboratory revealed the presence of sodium fluoride, a well known insecticide and ingredient of many roach powders.

In 1925 a group of poisoning cases was reported in a railroad shop, due to eating salad dressing. It transpired that in connection with labor disturbances, croton oil, a strong intestinal irritant, had been put into the salad oil. The dosage given was large enough to produce serious illness and several cases were in danger of a fatal outcome. All, however, eventually recovered.

Ground Glass.—Ground glass is sometimes accidentally or purposely introduced into foodstuffs. Several such instances have occurred in Chicago. Powdered glass, however, if ground fine enough, is not the deadly poison

it is popularly believed to be. An instance in point came to the notice of the writer several years ago. A package of self-rising pancake flour was brought to the Health Department laboratory, and found to contain 4 per cent of powdered glass. As soon as the analysis was completed the writer telephoned the interested person, advising that he see a physician at once. In a half-hour he walked into the office, very much frightened. "Did anyone of your family eat the pancakes?" he was asked. "Oh, yes," he said. "Is anyone sick?" "No," he answered. "How many cakes were eaten?" "Well," he said, "yesterday morning we had 'em for breakfast. My wife ate two; our little girl ate one; and I, not being very fond of pancakes, only ate nine. But they tasted a little gritty, so I thought I'd better have 'em analyzed."

And so goes, very briefly, the story of the chemistry of food control. Yet this science may be said to be only in its infancy. Research is constantly going on, yielding more knowledge on which to base food laws and regulations, improving and safeguarding the methods of food production and bringing out new foods, of which acidophilus milk, gelatin desserts and breakfast foods are instances. The next generation will see greater advances than the last and much of the credit must go to the patient scientist, puttering away in his laboratory, endlessly seeking the truth and as painstakingly finding ways of applying that truth to the problems of mankind.

Chapter VII—Article 3

THE SCIENTIFIC DISPOSAL OF SEWAGE

JOHN ARTHUR WILSON

Consulting Chemist, Milwaukee Sewerage Commission

Mr. Wilson has been chief chemist of A. F. Gallun and Sons Co., one of the largest leather manufacturers in the country, since 1916, after two years spent at the Proctor International Research Laboratory in Leeds, England. Both the preparation of leather scientifically and the proper treatment of sewage in filtering plants are very largely problems of *colloid chemistry*—that is how Mr. Wilson has been able to contribute so effectively, as consulting chemist, to the work of the Milwaukee Sewerage Commission, known throughout the world.

The sewage of any community may become a serious menace to public health, if not properly handled. With increasing size and congestion of cities, it becomes increasingly difficult to safeguard the people against diseases originating in sewage and often local conditions aggravate these difficulties. One of the most perplexing situations confronting any large city was recently met by Milwaukee after years of intensive research work by chemists and engineers. Its new sewage disposal plant will stand as a monument to scientific research. This chapter tells how Milwaukee succeeded in converting a waste which was offensive and prejudicial to health into an attractive and valuable fertilizer.

Milwaukee is more fortunate than many cities in having the great body of Lake Michigan at its very door as the source of its water supply, but less fortunate, perhaps, in having no place other than Lake

Michigan into which to empty its sewage. It became more and more painfully evident each year that the city could not go on dumping sewage indefinitely into its water supply without some day paying a terrible penalty. But there was no other practical way to dispose of the huge volume of waste waters constituting its sewage, because of the city's peculiar location. The only way out of the difficulty was to find some means of purifying the sewage before emptying it into the lake. This in turn involved finding also a method for reducing the materials drawn from the sewage to a condition permitting their economical disposal.

When the Milwaukee Sewerage Commission was organized in 1913, no method for accomplishing this was known. By the end of 1919 Chief Engineer Hatton had demonstrated conclusively that the newly discovered activated-sludge process of sewage treatment could be relied upon to give an effluent pure enough to be run into the lake safely at all times of the year; but the material removed from the sewage was not dry solid matter, but a soupy sludge containing about 98 per cent of water, which resisted further removal of water by any method then known to be practical. About 1,275,000 gallons of this sludge are obtained from the daily flow of 85,000,000 gallons of sewage from the 575,000 inhabitants of the district served. There is no place near Milwaukee where such quantities of sludge of this character could be thrown to waste. It was essential to the operation of the plant that this material be freed from water in a practical manner, although all previous attempts to accomplish this had met with failure. After years of intensive engineering and chemical research, involving every operation from the initial

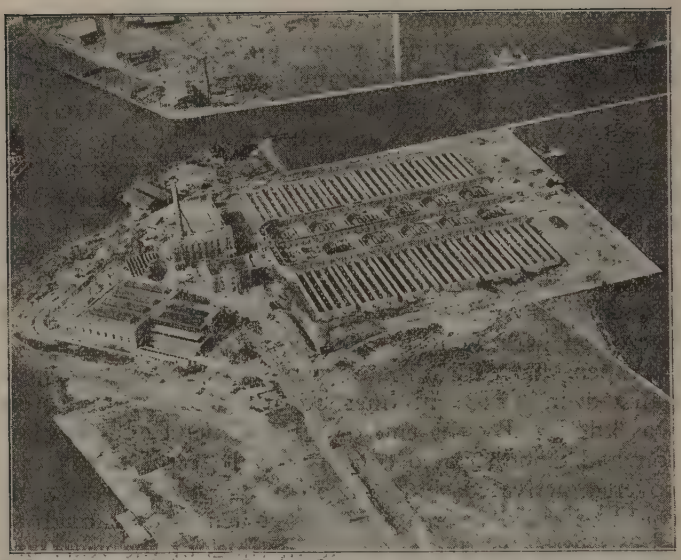

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FIGURE 1. AIRPLANE VIEW OF THE MILWAUKEE SEWAGE DISPOSAL PLANT.

Taken August 14, 1924, before the erection of the sludge storage house and before operation had been begun. In the right half of the picture are the aeration tanks separated into two groups with the settling tanks between them. The building to the extreme left is the filter house and the drier house adjoins it to the right. Just above the filter house is the battery of grit chambers, to the right of which is the power house. The building in front of the power house is the chemical laboratory, which also houses the fine screens. The experimental plant lies between the laboratory and power house.

treatment of the raw sewage to the effect upon plant life of the dried material as a fertilizer, a method was devised for reducing this sludge to an attractive dry powder having a high fertilizer value. It will be possible here to give only a brief description of the operation of the sewage-disposal plant finally built and of some of the fundamental principles involved.

The Water-Sewage Cycle.—The whole operation really begins with the pumping of the city's water supply from Lake Michigan. In serving the various sanitary and industrial purposes, the water becomes polluted with waste materials, which it conveys to the disposal plant, acting as a vehicle for carrying these wastes from many places to one designated point. It is now the function of the disposal plant to remove these waste materials from the water, sending it back to the lake essentially as pure as when first drawn from the lake. The disposal plant accomplishes its purpose in a number of different steps.

It will simplify the descriptions to make it clear at the outset that all operations are continuous; there is no stoppage of any part of the plant except for occasional cleaning or repairs. The sewage is carried to the plant at Jones Island by a system of intercepting sewers terminating in four siphons under the harbor entrance, 75 ft. below water level, the two carrying high-level sewage having effective diameters of 72 in. and 48 in. and the two carrying low-level sewage diameters of 54 in. and 42 in. The high-level sewage, about 80 per cent of the total, flows out by gravity, while the low-level sewage is pumped. The maximum capacity of the four siphons is 317,000,000 gallons daily.

Removal of Coarse Material.—All of the sewage

passes first through coarse bar screens which remove very large materials. The coarsely screened sewage then flows through grit chambers where the velocity of flow is reduced to less than one foot per second, permitting the heavier materials to settle out. The grit chambers consist of 8 open concrete channels capable of isolation from the rest for the purpose of draining and cleaning, which is done by means of a clamshell bucket operated from a locomotive crane.

The sewage then passes through the fine screens, consisting of 8 revolving drums. Each screen is equipped with 8 brushes for removing the screenings. The capacity of the 8 screens is 317,000,000 gallons daily with a 12-inch loss of head, or 149,760,000 gallons with an 8-inch loss.

Removal of Finely Divided Material.—The removal of coarse materials involves only simple and obvious mechanical operations, but sewage contains a much greater quantity of very finely divided material that will not settle out upon standing and that cannot be strained off even by ordinary filtering through cloth or paper. It is the removal of such material that requires the operation known as the "activated-sludge process."

The screened sewage is passed first into a mixing channel whose bottom is fitted with porous plates through which air is forced to keep the liquor above thoroughly agitated. At this point activated sludge returned from the system, to be described presently, is mixed with the sewage and the mixed liquor then flows on into the aeration tanks through feed channels, the rate of flow being controlled by sluice gates.

The aeration tanks consist of 24 rectangular concrete tanks, with 15 feet effective depth. In concrete containers

resting on the bottom of each tank there are 2,514 filtros plates, each 1 foot square. Air compressed to 10 lbs. per square inch is supplied to each container to be diffused through the filtros plates and allowed to bubble up through the mixed sewage and sludge as the mixture passes through the aeration tanks.

The flow is so regulated that it takes 6 hours for the mixed liquor to travel through the aeration tanks, during which time 1.5 cubic feet of air have been bubbled through it for each gallon of raw sewage entering the system. From the aeration tanks the mixed liquor flows into large sedimentation tanks, of which there are 11. Mixed liquor enters each tank continuously, the sludge rapidly settles to the bottom, where it is being drawn off continuously, while the purified water overflows continuously into the effluent troughs at the top of the tank and from thence through concrete conduits to the lake outfall. The effluent is clear and has been freed from about 99 per cent of the pollution originally present in the sewage.

The sludge drawn off from the bottoms of the sedimentation tanks, by means of the hydrostatic head, contains about 2 per cent of solid matter. It flows through conduits to a sludge well, from which about 90 per cent is pumped through a 48-inch cast-iron pipe, equipped with a venturi meter, to the channel where it is mixed with incoming raw sewage. The sewage contains about 250 parts per million of suspended matter and enough returned sludge is added so as to increase this value ten times in the mixed liquor before it starts its journey through the aeration tanks. The 10 per cent of the sludge remaining in the sludge well represents the increase in volume of sludge derived from the sewage,

which must be removed from the system and disposed of economically. It is pumped through an 18-inch cast-iron pipe to the filter house for the removal of water.

Mechanism of Aeration.—A better appreciation of the mechanical operations used to remove the finely divided material from the sewage can be gained from a study of the fundamental principles involved. When insoluble materials are suspended in a liquid, the rate at which they will settle depends upon the size of the individual particles. Other things remaining constant, the greater the size of the particle the greater will be its rate of settling. The tiny particles present in raw sewage which will not settle at all can be made to settle rapidly by causing them to unite with each other to form relatively large particles.

Much of the finely divided materials of sewage originally consisted of animal or vegetable tissues, presenting a relatively coarse structure. But through putrefaction and other chemical changes, these tissues have broken down into particles which will not settle out from the water upon standing. When oxygen is bubbled through neutral sewage, these tiny particles unite with each other, tending to form coarse particles analogous to the original tissues. If the bubbling of air is continued for several days, the particles become large enough to settle out fairly quickly upon standing.

If some of this settled sludge is now added to another portion of raw sewage and oxygen is bubbled through it, the tiny particles tend to combine with the larger ones rather than with each other. But the large particles are already of sufficient size to settle rapidly when the bubbling is stopped. Where two tiny particles combine, the double-size particle is still too small to settle out, but

where one large and one tiny particle combine, the resulting particle will settle at least as quickly as the original large one.

This explains why it is desirable to mix sludge already capable of settling rapidly, and which is called "activated sludge," with the incoming raw sewage before aerating it. The time required to bring the sewage to the point where purification can be effected by settling is thus reduced from several days to six hours or less.

One may liken the action in the aeration tanks roughly to the building of tissues in the animal body. The food is broken down in the animal's digestive tract into more finely divided materials, which pass into the blood streams. In the presence of oxygen, entering by way of the lungs and blood streams, these finely divided materials are rebuilt into tissues. In this operation, the tiny particles tend to fix themselves to tissues already built rather than merely to combine with each other. If the blood becomes much more acid or alkaline than normal, tissue building ceases. In the aeration tanks, the building of sludge proceeds at the greatest rate when the alkaline reaction of the mixed liquor is exactly that of the blood in the animal body ($pH = 7.4$). Upon the addition of acid or alkali, up to certain points, the building up of sludge stops and the particles begin to break down, just as tissues would do in the animal body under similar conditions. Fortunately, the mixed liquor in the aeration tanks tends naturally to maintain the reaction which is optimum for sludge building so long as air is bubbled through it, just as the blood in the animal body is kept at this same optimum reaction so long as the animal breathes.

The Sludge Problem.—The most difficult and serious problem encountered in the work at Milwaukee was to find a practical method of disposing of the 1,275,000 gallons of waste activated sludge produced daily. The success of the entire project depended upon devising some means of reducing this to a dry material that could be shipped and sold as fertilizer. But before it could be subjected to ordinary drying operations economically, it was necessary to reduce its water content from 98 to about 80 per cent by some kind of filtering device, which meant the removal from it of more than 1,000,000 gallons of water. Only then would it be practical to dry it further by the application of heat.

THE APPLICATION OF PHYSICAL CHEMISTRY TO THE PROBLEM

Degree of Dispersion.—From the standpoint of colloid chemistry, the problem of the activated-sludge process is one of regulating the degree of subdivision of the solid matter derived from the sewage and the degree of swelling or water absorption of the jelly-like constituents. In the aeration tanks, purification of the sewage is effected by the highly dispersed material joining the coarse, activated sludge, which settles quickly upon standing, thus permitting the clarified solution above to be decanted off into the lake.

The further removal of water from the settled sludge also depends upon the degree of dispersion of its solid matter. The particles formed in the aeration tanks may be large enough to settle sufficiently rapidly to permit the continuous purification of sewage, but yet too fine to permit the removal of water by ordinary filter-pressing, the finely divided matters clogging the filter cloth. The

building of sludge in the aeration tanks is a function of temperature; in the summer, when the temperature of the sewage was above 20° C., the sludge particles were built to a greater average size than in the winter, when the temperature of the sewage was below 10° C. Although the sludge formed at all times of the year was sufficiently coarse to settle rapidly and permit satisfactory and continuous purification of sewage, that formed in the winter was so finely divided that it could not be filter-pressed without special chemical treatment.

Part of the sludge consists of organized jellies, such as fibrous protein matter. The ease with which sludge can be freed from water by filter-pressing depends, not only upon the fineness of subdivision of a portion of the sludge, but also upon the extent to which the jelly constituents are swollen with water. A mass of fibrous protein matter can hold water in two very different ways: (1) in the interstices between the fibers, from which it may be forced out by the application of relatively slight pressure, and (2) within the substance of the fibers themselves, like the water absorbed by a block of gelatin, and from which the water cannot be removed mechanically except by the application of enormous pressure. The sludge will be in its best condition for filter-pressing when the individual particles are as coarse as possible and the water content of the jellies is at a minimum value. Happily, the chemical treatment required for maximum tendency of the sludge particles to unite to form large aggregates is the same as that required for minimum swelling or water absorption.

Effect of Adding Acid: the Isoelectric Point.—The particles of activated sludge drawn from the settling tanks are negatively charged electrically. This charge op-

poses the union of small particles to form larger ones, partly because of the repulsion of like charges and partly because the electrical charges increase the attraction of the surface of the particles for water. Moreover, the sludge is amphoteric; that is, it combines with acids forming positively charged particles or with alkalis forming negatively charged particles. By adding acid to the sludge, the value of the negative charge is decreased until it reaches zero, after which a still further addition of acid causes the particles to assume a positive charge of increasing value.

With increasing electrical charge, either positive or negative, the sludge particles tend to break down into smaller and smaller particles. Conversely, with decreasing electrical charge, the particles tend to unite, forming larger ones. An ideal situation is created by so regulating the acidity as to make the electrical charge equal to zero; that is, by bringing the sludge to its isoelectric point. Where acid or alkali is used to alter the electrical charge, the isoelectric point is usually recorded in terms of pH value, defined as minus the logarithm of the number of grams of hydrogen ion contained in 1 liter of solution. The isoelectric point of activated sludge as produced at Milwaukee was found to be pH = 3.4, or 0.0004 gram of hydrogen ion per liter. The curious thing about this sludge is that the isoelectric point was always found to be 3.4 regardless of apparent variations in composition of raw sewage from which it was derived. Adding enough sulphuric acid to the sludge to lower its pH value from 7.4 to 3.4 causes the particles to increase in average size and the sludge to filter at five times its normal rate. Another curious fact is that the rate of filtra-

tion of the sludge is multiplied by five, regardless of its rate before acidifying.

It so happens that even multiplying the rate of filtration by five is not sufficient to make the filter-pressing of winter sludge practical, and so other means of treating the sludge had to be found.

Effect of Adding Aluminum Sulphate.—The fact that the sludge particles are negatively charged made it seem likely that a coagulating effect and increase in rate of filtration must result from the addition of the right amount of aluminum sulphate, which, when diluted, gives colloidal alumina particles with positive electrical charges. The amount to use is that which will give alumina particles having the same total positive charge as the total negative charge on the sludge particles.

When this is done, the particles of sludge increase considerably in size and the rate of filtration is multiplied by eight. But for winter sludge, even this is not enough.

Effect of Temperature.—When a colloidally dispersed material is brought to its isoelectric point, coagulation is accelerated by the application of heat. Treating the sludge with acid to make pH = 3.4 and heating to give a temperature of about 90° C. multiplied the rate of filtration of the sludge by 30, making the filtration of even the poorest winter sludge a simple matter. Using aluminum sulphate and heat multiplied the rate of filtration by 40.

Thus methods were worked out for the chemical treatment of the sludge so as to make it easy and economical to filter-press any kind of sludge likely to be encountered.

Removal of Water from the Sludge.—The waste activated sludge from the sludge well flows first into a 42,000-gallon acidification tank at one end of which is a small control chemical laboratory. From a valve in the laboratory, the flow of sulphuric acid into the tank is regulated so as to maintain a pH value of 3.4 in the filter effluent. The tank is equipped with diffuser plates and baffles to permit thorough mixing of acid and sludge. If the condition of the sludge is very bad, a mixture of acid and aluminum sulphate is used.

The acidified sludge next passes into a heating system where its temperature is raised to the desired point. Much less heat is required for summer sludge than for winter sludge. The acidified and heated sludge is then delivered to a battery of 24 Oliver continuous vacuum filters. The hot filter effluent is returned to the heat exchangers where it gives up its heat to the incoming sludge. After giving up its heat, the filter effluent empties into the raw sewage line where it enters the aeration tanks.

The sludge cake, while still hot from the filters, is conveyed by belts to the driers, consisting of six Atlas direct-indirect-heat continuous rotary driers, each 7 feet in diameter by 60 feet long, enclosed in brick settings. Because of the tendency for the sludge to ball up and dry irregularly in the driers, an equal quantity of previously dried sludge is mixed with the sludge cake from the filters before entering the driers. This lowers the moisture content sufficiently to prevent balling. As the drum revolves, the hot gases from the furnace, mixed with large volumes of outside air, circulate around and into the drum through numerous air valves attached to the drum shell. About forty-five minutes is allowed for

the sludge to pass through the driers. The sludge cake coming from the filters has a water content of about 80 per cent. After it has passed through the driers, its water content is only about 5 per cent.

The dried material is conveyed to rotary screens, from which the finer siftings are transported by belt conveyors to a storage house with a capacity of 16,000 tons, equal to the output of the plant for about five months.

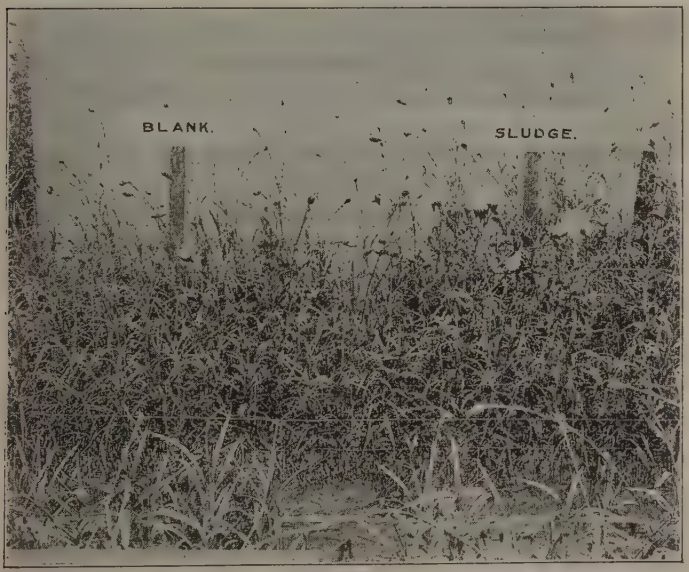

FIGURE 2. TEST SHOWING FERTILIZER VALUE OF ACTIVATED SLUDGE ON CARNATIONS. THE TREATED PORTION RECEIVED 75 POUNDS OF SLUDGE AND 30 POUNDS OF 24 PER CENT PHOSPHORIC ACID PER 500 SQUARE FEET. PHOTOGRAPHED AFTER 15 WEEKS.

The storage is made necessary by the seasonal market for fertilizer. The coarser particles from the screens are passed through a pulverator and then discharged into the wet-sludge-cake conveyor.

The Sludge as a Fertilizer.—Following is an analysis of a sample of dried sludge:

	Per cent
Water	5.75
Total phosphoric acid	2.34
Total nitrogen, as ammonia	7.32
Water-insoluble nitrogen	6.65
Water-soluble nitrogen	0.67
Active, water-insoluble organic nitrogen	3.94
Total available ammonia	4.61
Availability of nitrogen	62.97

When mixed with potash and phosphate, the sludge becomes a complete fertilizer.

V. H. Kadish and O. J. Noer have carried out very extensive experiments, which clearly demonstrate that such mixtures compare very favorably with the best commercial fertilizers on the market. Activated sludge is admirably adapted for use on golf courses in connection with top dressings for putting greens. It is also well suited to crops having a long growing season, such as corn and potatoes, and has been found valuable in greenhouses.

Future Work.—The plant is equipped with modern chemical research laboratories and a fine experimental sewage disposal plant where research chemists will continue lines of investigation already under way and strive to improve the operations of the big plant. Milwaukee has developed a really scientific method of sewage disposal which many other cities will copy. Its sewage is no longer offensive and a menace to health, but a source

of wealth to the community. It hopes to learn more of the basic principles of sewage disposal and to enrich the literature with data that will help all communities to deal intelligently with this difficult problem of sewage disposal.

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Chapter VII—Article 4

PROTECTION AGAINST INDUSTRIAL POISONING

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Dr. Hamilton is not only a pioneer in the investigation of industrial diseases in the United States but is also an international authority in this field, being one of the two American members of the League of Nations Health Committee since 1924. Dr. Hamilton was a special investigator of occupational poisons of the U. S. Bureau of Labor Statistics from 1911 to 1921, besides serving on Illinois State Commissions. Industrial labor has no stronger and no warmer friend than this champion of its right to protection against poisons.

The prevention of industrial poisoning involves two methods of procedure, one concerned with control of the work itself, the other with the personal care of the workers. The two will be discussed in this order.

MODE OF ENTRANCE OF INDUSTRIAL POISONS

In dealing with a poisonous substance in industry, from the effects of which it is necessary to protect the workers, the first thing to be determined is the mode of entrance of that particular compound into the human body. In ordinary non-industrial poisoning the toxic substance is usually swallowed, or it is inhaled as a gas, or rarely it enters through the skin, as, for instance, by inunction with mercurial ointment in the treatment of syphilis. In industrial poisoning the relative importance of these portals of entry is quite different.

Through the Air.—The most important mode of entrance is through the air which is breathed in and

which carries not only toxic gases but dust and fumes, the latter being really very fine suspensions of dust. These poisons, when breathed in, may produce a local effect on the upper respiratory tract, as do, for instance, caustic soda and potash, hydrochloric and hydrofluoric acids, and such irritating compounds as the chromates and arsenic trioxide, which cause ulcers in nose and throat. Other poisons which are non-irritating may, if in the form of gas, pass directly to the lungs and be absorbed. This class of poisons is very large, including such important ones as carbon monoxide, hydrogen sulphide, hydrogen arsenide, the petroleum distillates and many derivatives of the petroleum series, benzene (benzol) and turpentine. Brass poisoning occupies a special place, for it is caused by the action on the lungs of the zinc oxide fume which has been breathed in, but the latter is not a poison nor is it absorbed from the lungs. The finely divided zinc oxide powder spreads over the inner lining of the alveoli and by reason of its hygroscopic property it kills the lining cells and it is the resorption of these dead cells which sets up the chills and fever characteristic of "brass founders' ague."

If the poison is in the form of dust and fumes, the finer particles that are breathed in will in part reach the lungs, but the greater amount, including the coarse particles, will be caught in the saliva and swallowed. This is what takes place in poisoning from fumes of lead, dust of white lead, oxides of lead, or manganese dioxide.

The form of poisoning differs according to whether the poison which has been breathed in reaches the stomach with the saliva or passes directly to the lungs. In the former case it must meet with the defensive proper-

ties of the stomach, intestines and liver before it can reach the general circulation. If lead dust is swallowed, it is partly changed in the intestine to the sparingly soluble lead sulphide and passes out with the discharges. That portion that is absorbed goes to the liver, where it is in part or wholly eliminated through the bile and sent back into the intestine. The body has, therefore, a good deal of defense against such an attack, but if the lead is in the form of fumes or very fine dust it can pass in part directly to the lungs and easily reach the circulation, thus flooding the body rapidly with a large amount. This explains why acute, severe lead poisoning is seen only in occupations which expose a man to large quantities of finely divided lead dust or lead fumes.

Through Contact.—The second most important mode of entrance for industrial poisons is through the skin. A large class of toxic compounds used in industry, the coal-tar derivatives, which include nitrobenzene, aniline, trinitrotoluene, picric acid, phenol or carbolic acid, and the nitranilines and diamines, belong to this group. Others are mercury, carbon disulphide, which is used in rubber vulcanizing and in one kind of artificial silk manufacture, and methyl or wood alcohol. The fumes from all these compounds may cause poisoning and in every case of poisoning from contact there is, of course, exposure to fumes also, but practical experience shows that the most serious poisoning has followed the splashing of a liquid over the skin or soaking the clothes with it, or letting a powder fall on the sweating skin. In the case of mercury it seems probable that the fumes are more important than skin contact.

Contact with lead tetraethyl, the anti-knock compound used in gasoline, is particularly dangerous. Al-

though inhalation must also be carefully avoided, even more elaborate precautions must be taken against absorption through the skin because lead tetraethyl is dissolved by fats and hence passes easily through skin and tissues to vital organs, such as the brain. The regulations of the U. S. Public Health Service for the production and blending of tetraethyl lead with gasolene prescribe not only closed systems as far as is mechanically possible to prevent fumes and contact, but also provision for the immediate cleansing of the skin with soap and water after any accidental contact. Periodic medical examinations are also prescribed for all workers.

Through Contaminated Food, Etc.—The third and comparatively least important mode of entrance is by direct ingestion through the mouth, that is, by conveying the poison to the mouth from smeared fingers or contaminated food or tobacco or some implement of work such as a tiny paint brush. Instances of this form of poisoning are the handling of food by a painter whose hands are smeared with lead paint or by a lead worker whose hands are covered with lead oxides or white lead. It must be remembered, however, that these men are usually exposed to the much more serious danger of breathing air contaminated with lead dust, since they eat lunch only once a day but they must breathe as often as sixteen times a minute. The painter who uses white lead paint in interior work usually has to rub down with sandpaper or steel wool and it is the dust produced by this work which constitutes his greatest risk. An instance of direct swallowing of a poison is the work of the mechanical retoucher or commercial artist who uses a fine camel's hair brush with white lead paint to retouch photographs for reproduction, putting the brush

in his mouth to bring it to a point. A similar procedure is believed to be responsible for widespread poisoning among a group of women applying a radioactive compound to the dials of watches and clocks to make them luminous.

PROTECTIVE MEASURES

Protective measures, therefore, must be based on the characteristics of the given poison and the most important characteristic is its mode of attack on the body. Since, as we have seen, the larger number gain entrance to the body through the inspired air, it follows that the first element in protection is to prevent the poisoning of the air by gases or fumes, which are either accidentally produced during some process of manufacture or which are a necessary factor in these processes.

Accidental Formation of Poisonous Gases.—When conditions are favorable, gases may form which are not a normal part of the process and which are, therefore, unexpected and often undetected till their effect is shown by illness in the exposed men. Some of these gases are: hydrogen sulphide from decaying animal matter in tanneries, glue factories, soap manufacture; the formation of phosgene ($COCl_2$) from the use of carbon tetrachloride (CCl_4) as a fire extinguisher in an atmosphere poor in oxygen; and the formation of hydrogen arsenide in a multitude of processes where sulphuric or hydrochloric acid is brought in contact with a metal such as zinc, lead, antimony, which happens to carry arsenic as an impurity.

Prevention here means recognition of the possibility of such an accident and vigilance to guard against it. Hydrogen sulphide is likely to poison severely and acute-

ly or not at all, but hydrogen arsenide may cause a slow chronic form of poisoning, characterized by anemia, slight jaundice and dark urine. An alert physician can make the diagnosis sure by testing the urine for arsenic.

Escape of Gases, Fumes or Dust.—The second measure is to prevent the escape into the air of the working room of gas or fumes or dust which are formed in the course of an industrial process. In lead smelting, the dust from grinding ore, the dust and fumes on the feed floor, the fumes on the tapping floor and the dust in the great flues and bag house must all be prevented from contaminating the air which the worker has to breathe, and such prevention is perfectly possible by the use of proper mechanical equipment and, in the case of dust, of plenty of water. In steel mills the carbon monoxide so abundantly produced in the blast furnace was formerly allowed to escape at the top and top-filler furnaces were common. Now the furnace is mechanically charged and the carbon monoxide conducted through downcomers to be burned for the production of heat. This class of preventive measures constitutes much the most important steps in the prevention of industrial poisoning.

Respirators, Masks, Etc.—There are many industrial processes in which the escape of fumes and dust cannot be altogether prevented; others in which it is impossible to prevent the workers from coming in contact with poisons which enter through the skin. In such cases individual measures of protection must be used. If there are dangerous gases or fumes, some sort of filtering apparatus may be used. The ordinary rubber snout respirator is often of dubious value except when the poison is in the form of rather coarse dust. If filter paper is

used instead of a sponge the protection is greater, but the man may have more diffculty in getting all the air he needs. Undoubtedly such a respirator will filter out part of the poisonous dust and therefore lessen the danger, but it should never be regarded as a complete protection against dust and it is no protection against gases and little protection against fumes. There are now many forms of army gas masks provided with canisters containing appropriate filtering material and the Bureau of Mines is prepared to give advice to the manufacturer as to the proper filter needed for various poisons. The objection to the use of such masks is that if the man must do much physical work he will not be able to get enough air through the mask unless he is very persistent and patient and gradually accustoms himself to it. For this reason the respirator with hose attached supplying fresh air at a moderate pressure is usually preferred. The objection to this is that the man does not like to drag the hose around as he walks.

Ordinary snout respirators have provided a good deal of protection in white lead works and in lead oxide grinding and packing, in bolting and weighing litharge for rubber compounding, or mixing oxide paste in making storage batteries, but the presence of arsenic in dust makes it necessary to use a mask or helmet, for the dust would collect around the edge of the respirator and the arsenic would make the skin inflame and ulcerate. This is true of the flue dust of the Montana copper smelters, of the Utah and Colorado lead smelters and of the dust in Paris green manufactories. Army gas masks are especially valuable in plants where emergencies may arise requiring a short exposure to poisonous fumes, such as carbon monoxide in steel mills, or hydrogen sulphide

in making sulphur dyes, or benzene in by-products plants, or ammonia in refrigerating establishments, or nitrous fumes in the production of nitric acid and nitrocellulose. There are certain industries also with regularly recurring processes requiring unusual exposure and, therefore, unusual protection, such as the emptying of the calcium chloride chamber where the chlorine gas is held in loose combination with powdered lime, the cleaning and repairing of lead furnaces, the cleaning and repairing of quicksilver furnaces and the emptying of quicksilver condensers.

Resuscitation.—In addition to the provision of a gas mask or positive pressure respirator for emergencies, another precaution is needed whenever there is risk of poisoning by an asphyxiating gas, of which carbon monoxide is the most common. This is a supply of oxygen (95%) with carbon dioxide (5%) in a suitable apparatus,* for the experiments of Yandell Henderson of Yale, confirmed by many others, show that it is not enough to administer oxygen to victims of gassing, there must also be a stimulation of respiration such as is brought about by carbon dioxide. In no case should a pulmotor or lungmotor be used. The procedure advocated is the administration of the mixed gases, together with the prone pressure method of artificial respiration when this is needed. Almost always victims of gassing

*The Commission of the American Gas Association, composed of Drs. W. B. Cannon, Lawrence J. Henderson, Cecil K. Drinker, Francis W. Peabody and David L. Edsall of Harvard, with Drs. Yandell Henderson and H. W. Haggard of Yale, examined and accepted three appliances for the administration of this gas mixture. These are: the H-H inhalator, manufactured by the Mine Safety Appliances Co., of Pittsburgh; the Industro Oxygenator Universal Model, of the Protecto Safety Appliance Co., Newark; and the Atmos Inhalator, Model C, of the American Atmos Corporation, New York City.

are breathing somewhat when found, but victims of electric shock may not be, even when the heart is still beating. Such persons have only about ten minutes to live unless breathing is reestablished, and with them artificial respiration must be begun instantly, but its progress will be hastened by the use of the mixed gases as soon as the apparatus can be brought into play.

Prevention of Skin Contact.—When the danger comes from contact with poisons which produce their effect upon the skin or which make their entrance into the body through the skin, additional precautions are needed. Here it is necessary to minimize contact as much as possible, first by keeping the air clean. Trinitrotoluene dust in shell loading plants during the war caused much poisoning, especially during hot weather, when it fell on the exposed sweating skin of the workmen. Cleanliness of benches and apparatus, the substitution of pumping and mechanical conveying for hand work, of closed for open apparatus, are essential in such industries, for if the workmen must handle poison-smeared objects sickness will follow. It is also important to provide the workers with full suits of washable clothing, washable caps and socks, impervious footwear, and, if possible, impervious gloves. It must always be remembered that if the covering for hands and feet is not really impervious, it had much better be rejected altogether because if it lets in the poison it will act as a poultice and do distinctly more harm than good. Sometimes, when it is an emergency job, the skin can be completely covered. The man can wear a canvas helmet with isinglass eye holes and breathe through a respirator. This is done in cleaning flues and bag house tunnels filled with lead and arsenic oxides. Still better is the wearing of a helmet

with an air pipe, as is said to be done in the Anaconda copper smelters where the dust contains large quantities of arsenic. If the work must be done as routine, the man may be protected by smearing the exposed parts of the face with some bland ointment and plugging the nostrils and external ears lightly with absorbent cotton. This is done by packers of Paris green. In one factory producing coal-tar intermediates, including dinitrochlorobenzene and paranitraniline, both of which are very irritating to the skin and produce, when absorbed, systemic poisoning as well, the men were given each morning a suit of clean underwear, a suit of clean overalls, clean socks, wooden sabots, a clean canvas cap and, at the end of the day's work, were required to take a full shower bath. For emergency work such as cleaning and repairing nitrobenzene tanks and aniline reducers, emptying mercury condensing chambers, etc., the men should be required to strip and take a full bath when the work is over, using for the first-named compounds not only plain but acidulated water to help in removal of the oily compounds.

Cutting Oils.—There are two other classes of industrial poisons which have a very special action on the skin. The first class consists of cutting oils which in machine shops sometimes give rise to a great deal of annoyance and increased labor turnover because of epidemics of boils and furuncles. Marvin Shie, of the U. S. Public Health Service, found that such oils, which are used over and over again, may become heavily contaminated with suppurative organisms, the most serious of which is staphylococcus aureus. The R. F. Houghton Company of Philadelphia has found that so-called paraffin oil, which is the last oil expressed from solid

paraffin, is the one forming the most favorable culture medium for these organisms. The prevention of what is called oil folliculitis or furunculosis consists, therefore, in selecting the proper oil and in keeping it as clean as possible, occasionally subjecting it to heat sterilization, since the addition of chemical sterilizers has proved unsatisfactory.

Besides this, it is necessary to provide clean waste or cotton towels so that the men will not wipe their arms and hands with ordinary waste which often contains tiny particles of sharp metal, for these produce little cuts that offer an easy entrance for dirty oil. Trade eczema is often found in men who handle dyes which are not poisonous, but which make it necessary to use very vigorous cleansing methods which roughen the skin and deprive it of its normal oils. The same thing results from handling fat solvents, such as petroleum or benzene, methyl alcohol or other alcohols. Prevention consists in using bland soap mixtures and in anointing the hands with some animal fat or with castor oil.

Tar and Paraffin.—The second class is of great importance in Great Britain and on the Continent, where artificial fuel is made in great quantities, but of slight importance in the United States. This is the class including the so-called tar or pitch ulcers which later may become cancerous. It is not yet known just what compound in tar and pitch is responsible for these lesions, but, whatever it is, it is present in the products both of petroleum and coal tar distillation, although probably the latter contain the most. The lesions are slow in developing and the cancerous degeneration comes on slowly, is fairly benign, and rarely causes secondary growths. There is no method of prevention except by

protecting the skin against contact, and so far this has not been successfully accomplished.

Prevention of Slow, Chronic Poisoning from Gas.— In certain industries there are processes involving a continuous exposure to small quantities of volatile poison, too small to cause serious illness except after long exposure. Workers can hardly be expected to wear gas masks under such circumstances and, as we have seen, respirators are of little use in protection against a gas. Instances of this sort of work are: the making of artificial silk by the viscose process, which uses carbon disulphide, or by the lustron process, which uses tetrachlorethane; the vulcanization of rubber by Parkes' process, which uses carbon disulphide; spreading or dipping or cementing rubber with benzene as a solvent; making and spreading fabrikoid, or artificial leather, with benzene as a solvent, and sealing sanitary food cans with rubber-benzene cement. In such cases it is always possible to lessen the danger by shortening the exposure. The work may have to go on for eight or even twenty-four hours, but the individual does not need to be exposed for more than two or four or six hours. A further precaution should always be used in such industries; namely, periodical medical examinations to detect the early symptoms of poisoning in time.

THE INDUSTRIAL PHYSICIAN

This brings us to the relation of the physician to the prevention of industrial poisoning. So far, all the protective measures have been such as the engineer or the works manager must install. It is the physician's duty to examine all applicants for work, to weed out those he believes to be below par, and select the healthy

ones who supposedly will have a normal resistance to the industrial poison to which they will be exposed. He should divide those he accepts into at least two classes: those that are fit to do any kind of work and those that have some physical handicap and should not be exposed to more than slight risks. His duty does not stop here. He must watch the newly employed for the first few weeks, in order to weed out the over-susceptibles, for in every group of men or women there will be some individuals who will prove to be abnormally sensitive to a given poison, and this sensitiveness cannot be detected before it develops. All that can be done is to recognize the symptoms when they first appear and shift the man or woman to other work.

Over-susceptibility.—This factor of over-susceptibility is extremely troublesome, but it must be faced and not ignored. It should be no harder for a works manager to accept the fact that lead poisoning is bound to occur in one of his workers every now and then, unless all possible precautions have been taken, than it is for him to accept the fact that if the city water is infected with typhoid bacilli somebody will get typhoid fever. He usually argues that if, out of twenty men all doing the same work, only two show lead poisoning, it means that something is wrong with them. Either they are drunkards or they eat the wrong food, but in any case he is not responsible. Yet he knows that an epidemic of typhoid fever never involves a whole town—rarely as much as even ten per cent of the inhabitants, but nobody would argue from that fact that the typhoid-infected water was not responsible.

The ideal of the industrial doctor is a working force composed of immunes, and he will therefore reject not

only those of poor physical stock but the youthful, because it has been abundantly shown that the younger the victim the smaller the dose of poison required to produce its effect. During the war the British found that the general mortality from TNT poisoning was between 25 and 26 per cent, but for persons under 18 years of age the proportion was six deaths out of nine cases. We found in our TNT plants a distinct oversusceptibility on the part of the younger men. For instance, in one plant there were 48 cases of TNT sickness in two groups, one consisting of 29 men under 21 years, the other of 19 men over 30 years. The older men had averaged 49 days of work before they fell ill, the younger men only 10.5 days. Similar results were found in another plant where the older men averaged 56 days' exposure, the younger 7.5 days. In these two plants the men under 25 years made up less than 40% of the force, but the 15 cases of most rapid and serious poisoning were all in men under 25 years, most of them being 21 years or less.

Protection of Women.—It would be a great risk to select young girls for work with benzene. This is a poison which injuries the blood-forming tissues, resulting in anemia, leucopenia and a loss of the clot-forming constituents of the blood. Out of 37 severe cases of chronic poisoning in the literature 27 were in young persons, and out of 21 deaths 17 were in this age group. It is obvious that benzene poisoning with its tendency to hemorrhage must be especially dangerous for menstruating girls and pregnant women.

The question of the employment of women in poisonous work is still controversial. British statistics, confirmed somewhat by the scanty statistics collected in

the United States, point to a greater susceptibility to lead on the part of women as compared with men. Thus, a study of the pottery trades by the U. S. Public Health Service, published in 1921, showed that the average length of exposure of the male cases of lead poisoning was 17 years, of the female cases 9.3 years. Comparing the male and female dippers' helpers, the rate of lead poisoning was 8.4% for the men but 14.4% for the women. Moreover, women are more liable to that serious form of plumbism known as "lead encephalopathy," with convulsions, delirium or insanity, coma and sometimes death. Early statistics showed that from English potteries 34.9% of the women with lead poisoning had this form of the disease while 15% of the men had it. The figures from our potteries were one case in 17 among the men, one in 4.5 among the women. In the German munition plants the women employed in handling dinitrobenzene were of average physique while the men were those unfit for military service or invalided home, yet the women had a rate of poisoning of 66%, the men only 56.7%.

Another question to be considered in connection with the employment of women in poisonous work is the possible effect on their offspring. Although there is evidence that lead poisoning in the father results in injury to the offspring, the evidence is far clearer in the case of the mother.

Other Factors.—The industrial physician should also take race into consideration in the selection of his workers. Negroes are far more resistant to those poisons which affect the skin than are white men, but on the other hand they are more likely to suffer from lead encephalopathy than white men and are supposed to be more

susceptible to lead, although this last has not been statistically demonstrated.

The physician should concern himself with hours of work, for, obviously, if a man is exposed for eight hours he will absorb more poison than he will in six hours, and he will also have lessened the time for elimination. If a worker is to be protected against the cumulative effect of a poison he must be able, during the hours between his shifts of work, to get rid of all the poison he has absorbed. The English found during the war that it was a measure of economy to give from time to time a three-day holiday to their TNT workers in order to allow them to get rid of the poison and start afresh.

The physician should also see to it that workers exposed to poisons have proper food at least while they are in the plant. It is customary in Germany and in England to give workers with poisons, especially lead, a cup of milk or cocoa either when they start work or in the middle of the morning and afternoon, because abundant experience confirmed by laboratory experiments has shown that the presence of protein food in the stomach, especially milk, is an important protective measure against lead poisoning. The noonday meal should not be a cold, soggy, indigestible lunch from a lunch pail, but should consist of at least one hot, appetizing, nourishing dish.

The influence of alcohol on industrial poisoning is not so great as is generally supposed. Nevertheless, it is of some importance, especially in connection with certain poisons. Those which act on the central nervous system are, of course, helped in their action by alcohol, which is also a poison to the central nervous system.

Such poisons are TNT, ether, aniline, carbon tetrachloride, all of which have been shown by experience to have their action enhanced by alcohol. The tremor of mercurialism is worse in an alcoholic and so are the changes in the blood vessels caused by lead. Nevertheless, some of the severest cases of lead poisoning occur in men of entirely temperate habits.

Heat and humidity add greatly to the danger of those poisons which act on the skin or enter through the skin. It is possible also that the increased fatigue caused by work in hot and humid air may make men succumb more rapidly to any form of poisoning. Certainly it is true that moist heat is one of the most unhealthful features in industry.

Another very important duty of the industrial physician is to instruct the workmen in the measures of prevention which need their cooperation if they are to be successful. To do this he must explain the nature of the danger and the character of injury it may produce, as well as describing the precautions which the men must take. It is a great mistake to think that instruction of this kind frightens the workers and makes them imagine that they are ill when they are not. The reverse, rather, is true. If the whole question is ignored by the management and the pretence is made that there is no poisonous process in the plant, the men will not believe it, for they will learn from the older workers of instances of sickness that have developed, but instead of recognizing the real dangers and guarding against them they will be vaguely apprehensive and will be likely to attribute every ailment they have to their work although in reality the latter may have nothing to do with it, nor will it be possible to make them take the necessary pre-

cautions unless the whole matter is clearly explained to them.

Periodic Examinations.—Perhaps the most important function of the industrial physician is the periodical examination of all those who come in contact with poisonous compounds. The frequency of such examinations depends on the danger of the work. In British white lead plants, and in most American, the examination is weekly. In some American storage battery works the men are examined weekly, in others fortnightly, in others once a month. In connection with some poisons it is necessary to make not only a physical examination, but a microscopic examination of the blood. Thus, in a factory which makes artificial silk with the use of tetrachlorethane it has been the rule for over three years to make periodic examinations of the blood of those exposed to fumes from this compound, because it was found that the appearance in large numbers of a certain kind of leucocyte which is normally rare could be depended on to give timely warning that an attack of poisoning would occur unless the worker was temporarily shifted. In work requiring benzene the proper protection of those exposed involves from time to time a white blood cell count, for it is the fall in the number of these blood cells that gives the earliest indication of benzene poisoning. Examination of the blood for stippled red cells as an early indication of lead poisoning is practiced by many industrial physicians to confirm an uncertain diagnosis.

In a large British dye works, where accidental formation of hydrogen arsenide may occur without the management being aware of it, the physician makes his diagnosis between aniline poisoning (which is relatively

mild and brief) and the more serious poisoning from arsenic gas by examining the urine for arsenic. In some cases, such as chronic carbon monoxide poisoning or chronic naphtha or gasoline poisoning, the symptoms are not characteristic and it is well to err on the safe side and suspend temporarily or shift to other work those individuals who seem to be suffering from loss of health without distinctive symptoms.

It must be remembered always that although the acute cases of poisoning in industry are the ones that attract the most attention, the chronic are the most important. For one case of acute benzene poisoning with loss of consciousness and threatened instant death there are many cases of chronic benzene anemia. Moreover, if the victim of acute poisoning does not die quickly he will recover with no permanent damage, while the latter is suffering from serious injury to his blood-forming tissues. A man with an acute case of lead poisoning presents an alarming appearance, but under proper treatment may recover completely, while the one with a slow, chronic form, who may never have had an attack of colic, may be suffering from general arteriosclerosis with premature senility, quite incurable. It is the small, repeated dose of poison that produces lasting damage and requires eternal vigilance on the part of the management.

Future Progress.—Chemistry and medicine have thus made possible real progress in the protection of working men and women against industrial poisons, aided by legal regulations as well as by humanitarian good-will and economic self-interest on the part of employers. Much remains to be done in this field even in the light of our present knowledge and greater progress will be

made possible in the future through advances in chemistry. For instance, substitutes which are relatively nontoxic may be found to take the place of toxic compounds now in use. An instance of such substitution is the nonpoisonous titanic oxide, which has been made available to replace lead in paint. There is an ample supply of titanium and such a substitution would at a stroke remove one of the chief sources of industrial plumbism in this country. Another is the substitution of toluene (toluol) for the much more toxic benzene (benzol). At present this is hampered by the greater cost of toluene. A few years ago tetrachlorethane produced cases of severe or fatal toxic jaundice among men and women applying cellulose acetate coatings, but chemists discovered that a mixture of esters and ketones relatively harmless could be used instead. Thiocarbanilide has replaced aniline in rubber compounding, and perhaps the most beneficent change of all is the use of rubber latex instead of rubber dissolved in benzene for a sealing mixture.

We are still waiting for chemistry to find a safe substitute for benzene cement used to fasten leather to leather and leather to rubber; for wood alcohol in shellac, cements and dyeing artificial flowers; and for white phosphorus in fireworks, which has recently been found responsible for no less than 14 cases of phosphorus necrosis with two deaths among 71 employees.

Toxicology must join with chemistry in testing the new compounds which chemistry introduces into industry, so that rabbits and guinea-pigs may serve as experimental material. Had this been done by the producers and users of tetraethyl lead, they would have been saved an enormous financial loss and some 15 to 20 workmen

would not have lost their lives. In former years we depended on German investigators to make these experiments and publish them for the use of the world, but we can no longer look to their impoverished laboratories for this service; American investigators must now take it up. It is to be hoped that some agency under governmental control analogous to the Bureau of Standards will be entrusted with the duty of testing the toxicity on animals of new compounds and of determining the constituents of new mixtures.

Synthetic chemistry must have as one of its great objectives the further safeguarding of health and of life in the industries into which chemistry itself has introduced new poisons.

Chapter VII—Article 5

THE SAFEGUARDING OF DRUGS

PAUL NICHOLAS LEECH, PH.D.

Director, Chemical Laboratory of The American Medical Association

Dr. Leech has been an investigator in the chemical laboratory of the American Medical Association since 1913 and director of the laboratory since 1924. The courageous campaigns of the American Medical Association against worthless patent medicines and against every type of fraudulent medical claims as well as the constructive work of its Council on Pharmacy and Chemistry are well known; success in both activities has depended in large measure on the accuracy and reliability of the research work carried out in its chemical laboratory.

In Chicago hospitals over a hundred people a day are relieved of sensibility of pain by means of ethylene, a substance long used as an illuminating gas in railroad coaches. Recovery from the anesthesia, in the vernacular of the physicians, is "uneventful." This is made possible because chemists first have proved the absence of a possible dangerous impurity, carbon monoxide, in the anesthetic. To employ anything but the refined and carefully tested gas would be inviting disaster. For instance, in the city of Pittsburgh, not long ago, a well known surgeon was administering this gas before performing a major operation. He had used ethylene many times before, even though its introduction has been comparatively recent. The patient died on the operating table, evidently as a result of the anesthetic, and not of the operation. Another man died the next day. The particular brand of the product was just being introduced. Chemical aid and advice were sought immediately. Analysis of the gas revealed seven parts of

carbon monoxide in one thousand parts of the gas; at first glance, this appears to be a small amount, but when considered from the standpoint of its very poisonous properties, really a large amount, which accounted for the unfortunate accidents. In this case, the product was from a manufacturer who had not used adequate chemical control, and who probably did not appreciate the chemical precautions so vital to marketing products to be used for the sick. On the other hand, the fact that so many successful administrations of ethylene have been made—in one institution alone over 20,000—brings home forcefully the great protective value of the relatively few chemists engaged in controlling the purity of most of the ethylene produced before it is sold to hospitals. Furthermore, chemists working at the instance of the American Medical Association have devised tests and standards for "ethylene for anesthesia" so rigid that one part of carbon monoxide in 10,000 parts of ethylene can be quickly detected. Cooperating, manufacturers have agreed to market their brands of ethylene in accordance with these standards, and physicians, in turn, are using the acceptable brands, whose purity is controlled to the ultimate degree.

National Regulation; Pharmacopeias.—The safeguarding of drugs against such eventualities has had a long history. It was recognized years before the advent of scientific medicine that not only must the drugs be of constant composition, but deleterious contaminants should be avoided. Thus, in 1618 appeared the London Pharmacopeia which was mainly a compilation of formulas—detailing what ingredients to use, how much, and how to mix them, etc. The first Pharmacopeia in the United States was issued in 1821, and

unlike most of the previous pharmacopeias,* was national, so that all preparations prepared in different cities would be of the same composition. Since then, there has been compiled every ten years a new edition, including new drugs and omitting those which have fallen into disuse.

In the early part of the last century, the drugs were chiefly of plant origin; extracts of herbs, and later on, the principles themselves, such as quinine, from the dried bark of cinchona; morphine, from the poppy plants of India; and caffein, from tea or coffee. With the advent of the twentieth century, the list of drugs was being augmented by definite chemical compounds, complex in their composition, and, in general, synthetically made. Many of them were the results of the energetic work of the Germans on coal tar derivatives. The formulas calling for mixtures and decoctions of little-used herbs were rapidly going into the pharmaceutic scrap-heap. While all this was going on in the case of drugs sold for use of physicians, there was also developing a great evil of selling directly to the public all sorts of mixtures and decoctions, under various fanciful names, and with exaggerated claims of remedial actions, in other words, so-called "patent medicines."

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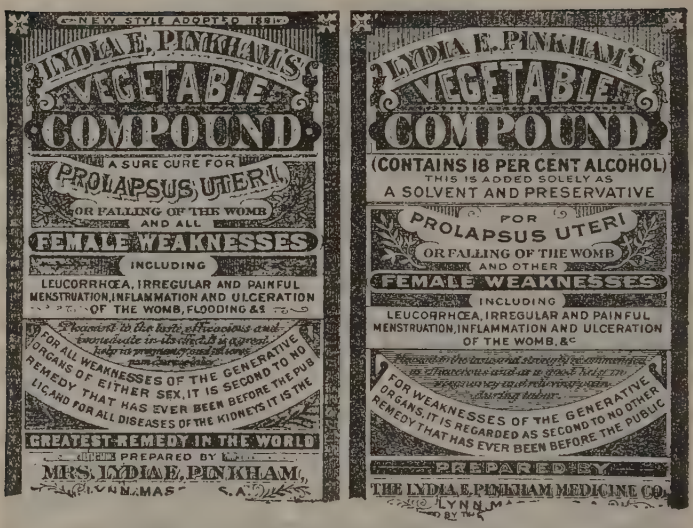
*A *pharmacopeia* (from the Greek *pharmakon*, "a drug or medicine," and *poieo*, "make" is, in the modern acceptation of the word, a book containing a list of products used in medicine, with descriptions, chemical tests for determining the identity and purity, and also formulas for certain mixtures of these substances. The necessity for such books is now recognized the world over, practically every progressive nation having adopted or legalized a book of standards. In those countries where there is not a national pharmacopeia, the standard of some other country is used. For instance, the U. S. Pharmacopeia has been translated—by private enterprise—into Chinese. For a popular history of pharmacopeias, and of pharmacy and alchemy, see *Four Thousand Years of Pharmacy*, by C. H. La Wall.

"PATENT MEDICINES"

The name "Patent Medicine" is a misnomer. While many of the newer drugs sold to physicians have been patented, the opposite holds true as far as the nostrums sold to the public are concerned. The reasons are obvious. If a substance is patented, its composition is known, its formula remains constant, and at the end of seventeen years, the protection of the patent ceases, so that anyone can manufacture the substance. The average so-called "Patent Medicine" is secret in composition, the formula or composition can be changed at the whim of the manufacturer without the ultimate consumer being any the wiser, and the proprietary rights are always invested with the owner of the trade-marked name. There have been a few examples of "Patent Medicines" which were really patented, for example, Castoria. Anyone can make Castoria, or sell it as that, as the patent expired long ago; but only one firm can make, say, *Fletcher's* Castoria, or *Smith's* Castoria. The name "Patent Medicine," therefore, as a rule, is vernacular for a nostrum advertised to the public. Before 1906 were the heydays of the "Patent Medicine" manufacturer. Habit-forming drugs, extreme curative claims, false labeling, all of these were unrestricted. Such conditions led to the formulation of the Pure Food and Drugs Act of 1906, one of the most beneficent statutes on record.

The Pure Food and Drugs Act.—This act was fairly inclusive. It prescribed certain rules and regulations for the marketing of remedies to the public. It recognized the U. S. Pharmacopeia and the National Formulary as official authority for the standardization and purity of

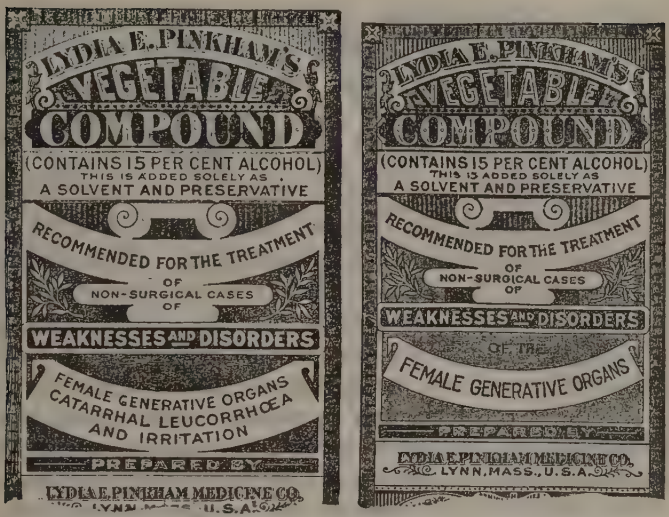
drugs sold to physicians; and, of course, it dealt with certain phases of food products. The law itself, however, would be totally incapable of enforcement were it not for the fact that chemists have perfected methods of investigation whereby the claims of composition can be verified—or exposed. The Government's Bureau of Chemistry has an ever-watchful force of "chemist detectives" trying to protect the nation's health in respect to remedies sold to the public. Yet this force, with its large laboratories and efficient as it is, is still insufficient. For the proper appreciation of the value of the Pure Food and Drugs Act, a few examples are needed. Briefly, then, will be discussed, first, its effect on "patent medicines," and second, its effect on drugs sold to physicians. In essence the law requires: (1) Substances sold directly to the public must be correctly labeled; for instance, not as "Syrup of Figs," but as "Syrup of Figs and Elixir of Senna," the latter being the essential ingredient. (2) The names of certain habit-forming drugs, such as opium, morphine, alcohol, acetanilid, acetphenetidin, etc., must be declared on the label. (3) Claims as to positive cure are prohibited unless it can be actually shown that the ingredients of the drug are capable of producing a cure—an unlikely possibility. Thus, in 1906, the public was enlightened to the fact that soothing syrups had contained morphine, that a whole host of elixirs contained alcohol, and Dr. Kilmer's Kidney Cure and all of the other "Cures" became, overnight as it were, "Remedies." Over a period of years, truthfulness in both advertising and on the label became somewhat a matter of relative honesty. An example of four stages of truthfulness is Lydia Pinkham's Vegetable Compound.



Four Stages of "Truthfulness"

STUDY THESE LABELS; THEY'RE WORTH IT. THEY TYPIFY THE CHANGES THAT HAVE TAKEN PLACE IN THE PAST FIFTEEN YEARS IN THE LABELING OF "PATENT MEDICINES." THE LABEL ON THE LEFT WAS ONE USED BEFORE THE FOOD AND DRUGS ACT WENT INTO EFFECT, THE HAPPY DAYS—FOR THE "PATENT MEDICINE" EXPLOITER — WHEN ANYTHING WENT.

The vigorous enforcement of the law by the Bureau of Chemistry soon began to have effect. Of course, the evil is far from abated, and probably will always be with us. But it is not the vicious crude affair of two decades ago.



NEXT IS THE LABEL USED AFTER THE FOOD AND DRUGS ACT BECAME EFFECTIVE, BUT BEFORE *THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION* HAD DEVOTED ANY LARGE AMOUNT OF ATTENTION TO THE "FEMALE WEAKNESS" CURES HUMBUG. THEN CAME THE THIRD LABEL FROM THE LEFT; THE LABEL ON THE RIGHT IS THE ONE NOW BEING USED. (FROM THE PAMPHLET "FEMALE WEAKNESS CURES" PREPARED AND ISSUED BY THE BUREAU OF INVESTIGATION OF THE AMERICAN MEDICAL ASSOCIATION.)

Needed Amendments to the Law.—The law, however, is still unsatisfactory. It does not include cosmetics; so anything can be sold across the counter for purposes of beauty, whether innocuous or containing dangerous mercury compounds in freckle removers, or

lead in certain hair dyes and even in some face enamel creams. Furthermore, there is no provision against the use of poisonous and potent ingredients, such as strychnine, arsenic, aconite, etc., in "patent medicines," the ultimate consumer having not the slightest warning of their presence. The only way the public has been informed of the danger often lurking in nostrums is the fearless publication of chemists' findings. This work of exposing the contents of many nostrums, which should be, it seems, a U. S. Government function, has been done by certain state laboratories, by the publication of articles, such as "The Great American Fraud," in *Collier's*, and mostly by the American Medical Association. A third defect of the law is that it is not up-to-date. Drugs which the public should not use except under the direction of a physician, have been introduced since 1906, and today are widely used without restrictions. Although codeine is prohibited, a drug which is considered far more habit-forming and the cause of many more accidental deaths, barbital (known also under the trade-marked name of veronal), or the related drug phenobarbital (trade-marked as luminal), can be incorporated in any proprietary remedy without its presence being declared on the label.

Education of the Public.—Due to the splendid work of the Bureau of Chemistry in prosecuting the more fraudulent "patent medicines," and also to the exposes published by certain altruistic agencies, the public is becoming more educated to the evils of self-medication with secret preparations. The public conscience has been awakened to such an extent that many firms, though

still in the "patent medicine" business, have considered it advisable to give the names of the ingredients—a few even give the quantities. Of course, there is a place for certain household remedies, and reputable pharmaceutical firms are supplying these drugs, which are generally simple in composition and whose purity is carefully controlled by competent chemists. Cod liver oil may be considered as one of the outstanding household remedies of today; yet it is valueless unless its vitamin content is assured. How much safer it is, therefore, to obtain a reliable brand, rather than a "patent medicine" emulsion of secret composition with no guarantee of potency or purity. It seems a pity that there must be "chemist detectives" to safeguard the sick and the well, but how vastly better it is that we have a Bureau of Chemistry and state laboratories than the situation is in some other countries where such welfare work does not exist. Chemistry, like all sciences, has a simple mission, namely, to establish the truth. The public can help by encouraging the work of these agencies so that public conscience, in the end, will be more and more awakened to the evils of "patent medicines;" and, in addition, to a proper appreciation of home remedies—the necessity of knowing their chemical composition and purity, and, equally important, to realize the limitations of self-medication.

OFFICIAL OR "STANDARD" DRUGS

The evolution of books of standards for drugs prescribed by the medical profession has been briefly discussed in the introduction. The book of standards of most interest to the readers of this chapter is the Phar-

macopeia of the United States.* That which makes the U. S. Pharmacopeia the peer of any in the world is that its standards are part of the law of the land. Drugs described in the Pharmacopeia are termed "official" drugs; they are sometimes spoken of under the ambiguous title of "Standard" Drugs. Before 1906, drugs were sold to physicians based on the specifications as then published in the current Pharmacopeia; there were reputable manufacturers who supplied official drugs, and dosage forms of them, which were reliable. There were also, unfortunately, many other pharmaceutical concerns which exploited the medical profession with unreliable materials. Mislabeling was not infrequent, and deteriorated products found their ways to the shelves. Today, this condition is quite different, and the story of U. S. P. drugs now is one of the bright chapters of pharmaceutical chemistry. As the law had given the government chemists the power to analyze the official preparations, and to prosecute firms whose products were found wanting, it was not long before the business directors of the pharmaceutical firms, particularly of the smaller houses, began to realize the need of scientific controls of their products. In other words, many of the houses which never had scientific staffs, sooner or later, as the case might be, established laboratories for both chemical and biologic assay of their products. This, in turn, has led to the creation of well-balanced scientific depart-

*The abbreviation for the United States Pharmacopeia is U.S.P. This designation is often seen in non-medical literature when referring to a U.S.P. drug. Its use by physicians and pharmacists is less common, as any official drug used by these professions must perforce comply with the Pharmacopeia. As the Pharmacopeia is revised every ten years, a Roman numeral is sometimes placed after the abbreviation to denote the revision or edition—thus, U.S.P.X, is the tenth revision, or eleventh edition.

ments in every large pharmaceutical house in America today. True, there are still some smaller concerns which cater mainly to the physicians who dispense their own drugs, which have not yet the services of an adequate scientific staff. But the handwriting on the wall is plain. Before very long, these firms will be forced either to engage the services of competent men, or more likely, to disband. In the case of most of the pharmaceutic houses, their large staffs of today are one of the great sources of protection to public health. These serious-minded men engaged in the ultimate interests of public health, are frequently the ones who have the foresight to develop new worthwhile additions to Materia Medica. The Pure Food and Drugs Act, while it has been of great direct benefit, has also been of immeasurable indirect benefit for the impetus it has given to the establishment of the research laboratories in our leading pharmaceutic houses of today.*

Laboratory Control.—As an example of the examining of products, take almost any described in the Pharmacopeia; for instance, acetylsalicylic acid (Aspirin). A representative sample of the product comes from the "factory" to the laboratory. Here chemists subject it to the specifications of the Pharmacopeia. It must have the right color, the correct crystalline structure, the stipulated solubilities, forming clear solutions in certain cases, and a melting point which indicates both identity and purity. It is then subjected to tests to rule out un-

*It is not to be inferred that there were no scientific staffs before the creation of the Pure Food and Drugs Act. One or two pharmaceutical houses did employ very competent staffs at that time, but the majority did not. All of them, however, have greatly augmented their research facilities, and with the advancement of biologic assay and laboratory medicine in general, no progressive firm could do without the provision in its budget for its scientific staff.

desired impurities, such as salts, resinous matter, free salicylic acid, etc. Finally, it is assayed for the quantitative content. The product must contain 99.5 per cent of acetylsalicylic acid. Only after complying with these tests can it be packed for the market.

Experience has shown that the widely used drugs of the Pharmacopeia are about as pure as can be; they are made in large quantities generally by a few firms, and distributed to others. The writer has often found the U. S. P. drugs to be amply pure for laboratory work and at times purer than the "chemically pure" substance of commerce—and less costly.

When it comes to the matter of tablets and pills—dosage forms—there is opportunity for error. It is little less than marvelous, therefore, to see machines turning out thousands and thousands of tablets with a variation of the active ingredient of not more than one or two per cent. This does not mean that every tablet on the market is accurate. The well-equipped manufacturer, with the watchful control laboratory, would indeed be chagrined to be haled before a court for misrepresentation of the declared amount. But there are, as stated previously, a number of houses whose history for scientific control and accuracy is not enviable, and probably there are others whose products would also be unreliable if the strong arm of the Government and the possibility of unfavorable publicity of the American Medical Association findings did not stand as a warning.

Before leaving official preparations, it might be well to point out that many drugs which at one time were protected by patent, are now official. But the trademarked name does not become common property in many instances. Thus, we have well-recognized drugs

PROPRIETARY
VS
NON-PROPRIETARY

Compare the relative Prices of

Identical Substances

sold respectively under

Protected and non-protected names

WHOLESALE DRUGGISTS' PRICES - Nov. 1927

Proprietary		*Non-Proprietary*	
Phenacetin	$0.63 oz.	Acetphenetidin	$0.22 oz.
Aspirin Bayer	0.85 oz.	Acetylsalicylic Acid	0.15 oz.
Veronal	3.00 oz.	Barbital	0.75 oz.
Atophan	2.75 oz.	Cinchophen	0.50 oz.
Duotal-Winthrop	1.07 oz.	Guaiacol Carbonate	0.30 oz.
Urotropin	0.60 oz.	Methenamine	0.16 oz.
Trional-Winthrop	1.90 oz.	Sulphonethylmethane	0.46 oz.
Sulphonal-Winthrop	1.70 oz.	Sulphonemethane	0.36 oz
Diuretin	1.85 oz.	Theobromine Sodium Salicylate	0.30 oz.
Aristol-Winthrop	1.80 oz.	Thymol Iodide	0.70 oz.
TOTAL	$16.15	TOTAL	$3.90

The total cost of an ounce each of these substances under a protected name is —— $16.15

The total cost of an ounce each of these substances under an unprotected name is —— $ 3.90

The cost of the proprietary name to the consumer is —————— $ 12.25

sold under two names—a proprietary and a non-proprietary one. The difference in the name is largely a matter of cost to the consumer.

The Chemical Laboratory of the American Medical Association has repeatedly analyzed the proprietary and official forms, and found them to be identical.*

NEW AND NON-OFFICIAL MEDICINES

In addition to official drugs, that is, those described in the United States Pharmacopeia and National Formulary, there are a whole host of non-official drugs. As no provision was made for standardization of these at the time the Pure Food and Drugs Act was passed, they are only of remote interest to the investigators in the U. S. Bureau of Chemistry. Mainly, this class comprises two types of drugs: The first type comprises remedies which have been introduced since the last revision of the Pharmacopeia, or had not yet gained sufficient medical recognition by the time of revision to warrant their inclusion in the book of standards. The second class includes proprietary products sold to physicians. In general, it may also be stated that there are two types of proprietaries, namely, those substances which have been patented and are really new discoveries; and, in the second place, proprietaries which are *mixtures* of substances and

*For a discussion, see:

"What's in a Name"—Editorial—*Journal of Industrial & Engineering Chemistry*, April, 1918, p. 255;

Editorial, *Journal of the American Medical Association*, April 13, 1918, p. 1097;

"Examination of Brands of Cinchophen," *Journal of the American Medical Association*, Dec. 5, 1925, p. 1829.

usually without much originality but kept alive by means of advertising.*

This latter group is easily disposed of. In the main, the preparations of this group are used by those physicians who have not yet recognized the irrationality of prescribing "shot-gun" preparations under proprietary names. This is not necessarily a severe criticism of these physicians, because many of them learned their Materia Medica in the days before there were available ready means of separating the good from the bad; when a complex prescription was a sign of good pharmaceutic art. Today, scientific therapy dictates simple prescriptions.

The Council on Pharmacy and Chemistry.—In about 1906, the same period in which the Pure Food and Drugs Act was under consideration, the advertising of proprietary remedies in the United States was indeed in a chaotic state. It is obvious that when one firm advertises a non-proprietary product, such as potassium iodide, it simply increases its sale in general; in other words, all the firms profit by this one firm's advertising. On the other hand, if a firm can sell a mixture of potassium iodide in syrup and water under a *protected trade-marked* name, such as "Iodo Syrup," then any increased sales from advertising will accrue directly to this firm, as no other manufacturer would have a right to

*This classification is general. There is also the case of drugs, described in the preceding section of this chapter, which were introduced years ago under proprietary names, and which still are sold under the trade-marked names, although the chemicals themselves have been recognized by the Pharmacopeia and are made now by other manufacturers. For instance, "urotropine" was introduced under the proprietary name "urotropine." The product itself is "methenamine," or, as chemists call it, "hexamethylenetetramine." It is described in the U.S.P.X as "methenamine." A physician or buyer may call for the product by its proprietary name—in which case only the special brand may be dispensed—or by its official name, in which case any brand of official purity may be dispensed.

market the same mixture under the registered name of "Iodo Syrup." In 1906, editors of medical journals were carrying advertising of proprietary polypharmaceutic mixtures of this type along with the worthwhile preparations, for just as surely as there are evils to proprietary medicine manufacture, there are also advantages. In this age of large overhead costs, which include the great costs of research, before a worthwhile drug can be developed and introduced by a pharmaceutic concern, such a drug must be introduced as proprietary to remunerate the manufacturer. It would have been wrong, therefore, for any medical authority, such as our leading medical journals, to have declared that all proprietary medicines are bad, as some of our very best advances in Materia Medica are the result of the introduction of new remedies as proprietary medicines. There was need, however, for some way of advising the medical profession concerning the honest and dishonest, the scientific and unscientific, in this large field of non-official drugs. To meet the situation, as it existed two decades ago, the American Medical Association created a Council on Pharmacy and Chemistry, composed of eminent chemists, bacteriologists, pharmacologists, clinicians, etc., connected with educational institutions and serving without remuneration, for the purpose of gathering and disseminating such information as would protect the medical profession in the prescribing of proprietary medicinal articles. In pursuance of this object, the Council examines the articles on the market as to their compliance with definite rules designed to prevent fraud, undesirable secrecy and such abuses as arise, for instance, from advertising directly or indirectly to the laity. In order to verify or disprove the composition of

remedies and to elaborate proper standards, there also was created the Chemical Laboratory, which subjects products to tests and elaborates standards. Such articles as appear to conform to the rules are accepted, and their essential features are described in the annual publication of the Council, New and Nonofficial Remedies, if they come within the scope of this book.*

In this book physicians can find descriptions of the composition, the actions and uses of new drugs, together with scientific and disinterested statements on the efficacy and promise of efficacy of the drugs as judged by the evidence to the date of publication; their advantages as well as disadvantages are impartially described. There are also valuable discussions of groups of related compounds and the probable value of therapy based on their use. To appreciate the service thus rendered by the Council, we must remember that almost numberless new remedies are being constantly introduced and to a large extent with the object of commercial exploitation; that manufacturers and discoverers are inclined to view results of preliminary trials through the rosiest of spectacles even in the best of cases, and at worst are occasionally tempted to make altogether unjustifiable claims for their products. Besides giving physicians through this annual publication direct information on new and non-official drugs, the Council serves also to protect the physician and the public through its influence on advertising. Practically every medical journal in America of the better class accepts no advertisement of a proprietary article which has not been accepted by the Council. Thus has been created an extra-legal method of pro-

*The rules of the Council, with explanatory notes, can be obtained by addressing a request to the American Medical Association, Chicago.

tecting still further the sick, and advancing scientific medicine.

The Chemical Laboratory of the American Medical Association.—To appreciate the value of drug standardization of non-official products, the alkaloid, ephedrine, offers an apt illustration. Ephedrine, extracted from an old Chinese plant, *ma huang*, was considered of little value until a few years ago research workers discovered that it possessed certain hitherto unrecognized properties of value in asthma, hay fever, and the like. This awakened a great deal of interest on the part of the medical profession, and soon demands were being made for the alkaloid salts. For a while, because of the heavy demand, the products were supplied from various uncontrolled sources. There was no assurance of definite purity; furthermore, because of the molecular constitution, there are four possible, closely related alkaloids, though only one, *levo*-ephedrine was desired. Into this situation, the Chemical Laboratory of the American Medical Association entered; it made a study of the drug and elaborated certain specifications. As soon as these standards were available, practically every manufacturer of ephedrine in the United States agreed to follow them. The significance is apparent. It means that the American public now has a drug of recognized purity, and that the physician on the eastern coast can obtain comparable results with the physician on the western coast, because the compounds are all of the same degree of purity and, therefore, potency. In the case of the product of one firm, it was necessary to make it 40% purer in order to conform to these new standards, but the firm could afford to do this because the competition with other firms was on the same basis. The medical profession also

safeguards itself by asking its laboratory to investigate any drug which a proprietor endeavors to force on the medical profession without telling its full composition. The Association's laboratory also frequently checks up on the preparations as found in the open market, thereby being in position to inform the medical profession concerning keeping qualifications, etc. This latter work of late years has been rarely necessary, because of the careful supervision by the scientific staffs of the progressive drug houses.

BIOLOGIC CONTROLS

The Hygienic Laboratory of the U. S. Public Health Service.—It would be misleading to leave the inference, however, that the safeguarding of drugs is done entirely by chemists. In many instances, the methods of chemistry, refined as they are, are still too crude to measure the potency of certain active principles. Thus, epinephrine (introduced as adrenaline), insulin, and extracts of the pituitary gland, are subjected by pharmacologists to animal tests. The data thus obtained are more valuable for a guide to clinical dosage than any chemical assay so far devised for this type of substances.

The important group comprising serums and vaccines are still beyond the ken of the control chemist. Fortunately, however, the reliability and potency of these biologic preparations (as far as interstate traffic is concerned) are carefully watched by the Hygienic Laboratory of the U. S. Public Health Service, with its corps of trained bacteriologists and associated professional men. The monumental work of the scientists of the Hygienic Laboratory has been of immense benefit to the citizens of the United States. The Service inspects the reliability of the firms and the competency of the work-

ers who prepare the commercial vaccines or serums; it investigates the premises, animal facilities, etc., of manufacturing laboratories, and checks up on the finished product in order to determine the accuracy of the declared statement of potency and the freedom from undesirable contaminants. Only after complying with the requirements can a license to manufacture and sell in interstate traffic be obtained. The work has served as a model for other countries, and is closely followed by the appropriate committees of the League of Nations.*

PRE- AND POST-WAR CONDITIONS

In discussing the drug industry and the reliability of drugs, it is almost necessary to refer to the pre-war and the post-war periods. Before the war, practically all the synthetic drugs used in America were made in Germany. It seemed for a while that if a new substance could not be used as a dye *per se*, it must be accorded a place in the ever-increasing list of coal tar preparations for sale to the physician. We owe a large debt to Germany for its work in synthetic drugs, for the greatest advances in recent times in new remedies for the sick have been in the case of preparations made from coal tar, but the commercial exploitation of the American physician was overdone. During this period of the German synthetics, America was not doing much as far as the manufacture and discovery of definite chemical substances were concerned; the American pharmaceutical houses were largely manufacturers of so-called standard products,

*In addition to biologic preparations, the Hygienic Laboratory also controls the toxicity of Arsphenamine ("Salvarsan"). While this product is assayed chemically, yet the possible poisonous impurities are such that they can be much more reliably determined by tests on animals.

pills, tablets, etc., or of proprietary mixtures.* Synthetic medicinal chemistry in America was neglected. Twenty years ago, in the case even of the remedies sold to physicians, frauds were not altogether uncommon; deception—unintentional or intentional—was rampant, and medical journal advertising teemed with statements concerning the composition of remedies which any chemist familiar with medicines would know were untrue. Most of the products which came from Germany were reliable, as far as chemical composition was concerned—more so than from any other country at that time. Many of them were standardized in the Laboratory of the American Medical Association. With the advent of the war, however, American chemistry, as has been told elsewhere, received a tremendous impetus; today, the conditions are almost the reverse of the pre-war period. American pharmaceutical houses are making valuable contributions in drug manufacture, and with the reputable firms, the products are of a high degree of purity, an evidence of their rigorous control. The new products offered resemble, in reliability, the German drugs of the pre-war period. To date, the American firm seemingly has learned from the lessons of the pre-war period not to flood the American market with too many synthetic drugs, as the Germans did previous to the war. The result has been that the American physician is not confronted with all sorts of new coal tar derivatives. American pharmaceutical houses are putting out fewer products with greater likelihood of permanent value, and improvement has been steady. In order to appre-

*For a discussion of this phase (within the last twenty years), see "Chemistry in the Service of Pharmaceutic Medicine," *Journal of the American Medical Association*, July 11, 1925, pp. 138-141.

ciate the safeguarding of these proprietary drugs made in America, one must keep in mind the fact that practically all of the worthy preparations (there are still many "non-accepted" brands) are under just as rigorous control as are the official drugs, and their standards are published currently, with such revision as may become necessary as a result of better manufacturing technique.

The names of a few of the important contributions made to chemotherapy on this side of the Atlantic must suffice to illustrate the new era. For instance, we have Kendall's thyroxine; Banting and Macleod's insulin (a Canadian contribution); tryparsamide developed at the Rockefeller Institute for Medical Research, an improvement on arsphenamine for the treatment of neurosyphilitic diseases, such as paresis; butyn, devised by Adams and Volwiler, an improvement, for use on mucous membranes, over the local anesthetic procaine; a number of antiseptics and germicides; Rowntree's phenolsulphonephthalein for the test of the functioning of the kidney and his phenoltetrachlorophthalein as a test of liver function; the complex iodine compounds which, due to researches of Graham, and of Whittaker, permit visualization of the gall-bladder; and among the biological products, the scarlet fever antitoxins of the Doctors Dick and Dr. Dochez; the separation by Kamm and coworkers, of solutions of pituitary extracts into two distinctly differently acting substances; the parathyroid hormone of Collip (of more academic than practical clinical importance); the liver diet therapy developed by Koessler, and by Minot and associates; the vitamin concentrates; erysipelas streptococcus antitoxin, etc., etc. A new era has indeed dawned.

CHAPTER VIII

The Alleviation of Suffering

ARTICLE 1

CHEMISTRY IN MEDICAL DIAGNOSIS

LEONARD G. ROWNTREE, M.D.

Mayo Foundation

Dr. Rowntree, a Canadian by birth and of Canadian training in medicine, found an opportunity for his active interest in medical research in the Johns Hopkins Medical School (1907-16). Then, after four years as professor and head of the Department of Medicine at the University of Minnesota, he joined in 1920 the staff of the Mayo Foundation, in Rochester, as chief of the Department of Medicine. Dr. Rowntree, besides authoritative work in varied fields of medicine, is the celebrated inventor of the phenolsulphonephthalein test of kidney function, an invaluable test for purposes of diagnosis and treatment and as a guide to a surgeon. This advance was followed by a successful search for other chemical indicators to be used to shed light, without operation, on the condition of other organs. He himself discovered a similar test for liver function and, following in his lines, others developed appropriate indicators (all "phthaleins") for X-ray examinations of the gall-bladder. Such tests, made without suffering and risk on the part of the patient, avoid exploratory operations and make for precision in diagnosis—a blessing to man.

LIFE is a chemical function. The living body is composed of innumerable cells, each a little chemical factory, carrying out its allotted task and making its own contribution to the substance and function of the body as a whole. To each cell the arterial blood brings water, salts, foodstuffs and oxygen, and from it the

venous blood collects products of the cell's activity together with waste substances.

In all but the simplest forms of life there is a division of labor, and each cell contributes that special activity which is essential to the association of chemical processes that constitutes the living individual. The energy of the body in its various forms is all derived from the chemical energy of the food. This transformation is brought about by the harmonious and interdependent action of all the cells of the body. The sum total of all these chemical changes, whether they lead to simplification of the molecule as in combustion and release of energy, or to complexity of chemical structure as in the manufacture of tissue capable of carrying on vital chemical function, is known as metabolism. The laws governing metabolism are predetermined, fixed and operate interminably throughout the ages. Individuals are but incidents in a chemical sense. Species are cast in various molds, generation succeeds generation and youth is followed by age, but through it all goes metabolism. Life persists and health obtains provided metabolism proceeds along normal lines. Slight variations result in illness, and marked aberrations end in death.

In health Nature is consistent: normal chemical processes result in normal function. Even in disease her ways are not haphazard; uniform alteration in metabolism will probably result in a uniform type of perversion of function. This may find expression subjectively in symptoms, and objectively in signs, and may be accompanied by correlated chemical changes which can be determined qualitatively or quantitatively in the laboratory. It is this consistency that makes diagnosis possible.

Diagnosis.—The doctor's success in diagnosis is de-

pendent upon his knowledge of Nature's method of response to various insults and injuries of all kinds, and upon his ability to assemble and evaluate the evidence of disease and to interpret it correctly. Three considerations enter into the making of his diagnosis: the history, or what the patient can tell of his illness, the physical examination, or what the doctor can learn directly from his five senses on examining the patient, and the laboratory study, or what the doctor can learn indirectly through the scientific investigation of the patient, of the tissues including the blood, the body juices and the excreta.

Diagnosis would not be so difficult, perhaps, were the causes of the disease always apparent. The patient comes complaining, not of a disease, but of symptoms. It is the doctor's function to obtain as accurate a story as possible, to find the signs of disease, and elicit such other evidence as is important in establishing the nature of the underlying defect. He should also seek its cause. Efficiency consists in determining the real nature and cause of the trouble, with the minimal expenditure of time and energy. Sometimes the diagnosis is best achieved through a careful analysis of the history, sometimes by prolonged observation and repeated physical examinations, and at other times through one or more laboratory tests. But in certain obscure cases all these sources of evidence must be taxed to the very limit.

A patient may, for example, consult his physician complaining of the remarks his friends have been making recently about the yellowish color of his skin. He has never had this peculiar color before, nor has any member of his family been similarly afflicted. His only complaint is that he is "lacking in pep." The doctor

now makes his examination and finds the skin and the eyes of this yellowish tint, and in addition a spleen that is somewhat larger than normal. The cause of the jaundice will be revealed only by further examination. It might be a simple case of catarrhal jaundice, or obstructive jaundice due to gall-stones within the duct, or a cancer of the head of the pancreas, obstructing it by pressure from without, or it may be due to quite a different cause, namely, the excessive destruction of the red blood-cells (the hemoglobin of which is the normal source of bile pigment), and the appearance of the excess in the skin and eyes.

In this case the history and physical examination fail to yield the diagnosis, and the assistance of the laboratory must be invoked. The urine contains urobilin, and the presence of adequate amounts of bile in the stool proves that the jaundice is not the result of complete obstruction of the bile duct leading to the intestine. The bile pigments in the blood are next investigated and are found to be increased quantitatively, let us say to three times normal. Furthermore, they give qualitatively a type of reaction which excludes obstruction of the biliary passages. Perhaps, then, the excess of bile in the blood is due to excessive destruction of the red blood-cells. In this connection, we must remember that the red blood-cells of the body are being constantly broken down even in health. If unduly fragile, they are found to disintegrate more readily than normal when suspended in increasingly dilute salt solution. By this method the fragility of the red blood-cells is then found to be decidedly increased. This furnishes the evidence that the jaundice is probably due to increased destruction of the red blood-cells. The spleen has been found

to be enlarged, and it is known that this is likely to occur when it is overworked in the destruction of blood-cells. As the result of these studies, the diagnosis of acquired hemolytic jaundice is made. The treatment carried out is based on this conception of over-activity of the spleen, which is therefore removed; the jaundice disappears and the patient recovers his usual health.

This is a common experience in any modern medical institution. With proper laboratory facilities, the chemical investigation aids in determining the diagnosis. The diagnosis made, treatment is instituted promptly, and within a short space of time the patient is well and returned to his normal environment. Thus chemistry not only serves the cause of humanity, but helps to conserve the most precious unit in our economic structure, the human life. Unfortunately all doctors are not equipped to carry out these examinations. But, on the other hand, a large and increasing proportion of physicians are in a position to carry out the simpler diagnostic chemical procedures in their own offices and to command more elaborate service as they recognize its need.

Big Returns from Small Investments.—Certain tests are so simple and yield information of such crucial importance that they have become a part of the "routine examination" of the patient. Accuracy demands them. Experience has taught us that despite the greatest amount of clinical acumen, certain diseases escape recognition at times unless routine methods are employed. The routine tests include an examination of the urine (urinalysis) and the study of the blood from the standpoint of the number of red and white cells and the concentration of the oxygen-carrying component in the blood. This is hemoglobin, the substance which gives the red color to

the blood. In addition, the Wassermann test for syphilis has proved so valuable that despite its technical difficulties, it is almost a routine in our leading hospitals and institutions. By these methods, the presence of serious diseases such as Bright's disease, anemia, diabetes or syphilis is often made apparent when the history and physical examination have failed.

URINALYSIS

Urinalysis is a very simple procedure, yet it has a very important bearing on diagnosis. The clues it affords are almost innumerable. It takes into account the amount of urine in twenty-four hours, its color, acidity, and specific gravity, the presence or absence of albumin and sugar and the forms of sediment recognizable on microscopic examination.

Normally, an adult voids about one and one-half quarts in twenty-four hours, but the amount may vary anywhere between one and two quarts. In disease, however, it may be diminished, no urine at all may be passed, or it may be increased even to 30 or 40 quarts a day. Large amounts may indicate diabetes mellitus, diabetes insipidus, vascular disease or Bright's or other forms of kidney disease; while decreased amounts may indicate obstruction in the urinary tract or forms of kidney disease or heart disease associated with dropsy.

The urine is usually yellow or amber in color and is also transparent. In disease, it may become turbid or vary in color from that of spring water to a white, yellow, greenish red, brown or even black. The color itself often suggests disease. At least twenty or thirty diagnostic clues are suggested solely by changes in the

color of the urine. Naturally, these are only clues and proof must be sought by other methods.

The amount of solid dissolved in the urine determines its specific gravity, which normally varies from 1.016 to 1.030. A dilute urine is encountered after excessive ingestion of water in diabetes insipidus and in some cases of nephritis, while a concentrated urine is found in the ordinary form of diabetes mellitus and in diseases of the kidney associated with dropsy.

Acidity.—The normal reaction of the urine is slightly acid, due to the presence of acid phosphates. In disease, it may become excessively acid, neutral or alkaline. The changes in reaction of the urine are detected in a most simple way through changes in the color of strips of litmus paper immersed in it. The urine becomes excessively acid in gout, diabetes, certain febrile states and in some forms of rheumatism and arthritis. Alkaline urine, on the other hand, usually suggests inflammation of the bladder or urinary tract or the ingestion of excessive amounts of alkali by way of medical treatment. These changes in reaction, in addition to their diagnostic importance, are related to the problem of the acid-base equilibrium of the body, a subject of very special interest, about which we will have more to say later.

Albumin.—A test for albumin is easily made in a test tube. When present, albumin can be roughly expressed as much or little. Albuminuria may be physiological in nature, the result of exposure or of a cold bath, or it may be due to faulty posture, as in orthostatic albuminuria. It may mean disease of the kidney, inflammation, as in Bright's disease, congestion as a result of failing heart, back pressure and obstruction of

the urinary tract, caused by enlargement of the prostate gland; or it may be the result of infection of the kidney as in tuberculosis, or perhaps tumor of the kidney or multiple tumors elsewhere in the body, especially in the bones. Its true significance can be determined by questioning the patient further, by repetition of the clinical examinations, by varying the conditions under which the urine is collected, and by qualitative and quantitative studies of the urine and blood. For instance, if albumin is absent while the patient remains in bed, but appears when he is up and about or standing at attention, it indicates, in all probability, orthostatic albuminuria, the more so if the patient is a rapidly-growing adolescent boy. This form of albuminuria is of no clinical significance whatsoever, and the albumin will disappear, in all probability, between the twentieth and thirtieth years. If, however, albuminuria is present continuously and is accompanied by casts and blood-cells in the urine, by dropsy, increase in blood pressure, and changes in the posterior wall of the cavity of the eye, it probably indicates Bright's disease.

It is, therefore, clear that it is the duty of the physician in every instance, on finding albumin in the urine, to determine its cause and its significance, and to discover the underlying pathological changes.

Sugar.—The next routine test of the urine is that for sugar. The presence of sugar does not, of necessity, indicate diabetes mellitus, since the sugar may not be glucose. It may be levulose, maltose or lactose, the latter appearing during pregnancy or during the period of nursing. For each of these sugars there are special tests.

Even when it has been decided that the reducing substance is glucose, the diagnosis of diabetes is not

established. It may indicate simple alimentary glycosuria, resulting from the excessive ingestion of carbohydrate, or emotional glycosuria caused by some undue strain on an unstable nervous system. It may indicate organic disease of the brain or prove to be merely renal glycosuria, an interesting clinical condition associated with abnormally high permeability of the kidney to sugar. But when the sugar persists in the urine and the sugar in the blood is found to be increased, when the patient presents the clinical manifestations of diabetes, and the output of sugar in the urine is increased with a diet rich in carbohydrates, the diagnosis of diabetes mellitus can be considered established and treatment may be instituted. Subsequently, quantitative chemical studies may become necessary. These will be discussed later.

Sediments.—Several chemical entities may be recognized in the urine by their characteristic types of crystals. Thus, crystals of uric acid, or urates, of phosphates and of calcium oxalate are found normally, and may be recognized at a glance. But in disease one may find at times cystine crystals, indicating a peculiar inherited perversion of protein metabolism, leucine and tyrosine crystals indicative of acute yellow atrophy, a fatal disease of the liver, or cholesterol crystals associated with the excretion of fat in the urine. Among the organized sediments of the urine, one may also find evidence of chemical products, red blood-cells and hyaline, waxy and fatty casts from the kidney tubules indicative of renal disease. With a special prism, doubly refractile lipoids are seen at times showing a dark central cross and bright peripheral quadrants. These suggest the presence of a syphilitic form of nephritis.

Urinalysis affords literally hundreds of clues to all

kinds and sorts of diseases, constitutional diseases as well as those of the genito-urinary system. From the standpoint of accurate diagnosis, the analysis of the urine is indispensable.

HOW THE KIDNEY BEHAVES IN DISEASE

During the last two decades we have learned some things of great importance, one of which is how to determine the function of the kidney in health and in disease. This is, of course, of untold value in the study of all its diseases. Many tests of renal function are now available, but information of crucial value can be obtained from a few very simple procedures.

The Phthalein Test or Red Test.—Phenolsulphonephthalein, a dye which is bright red in alkaline solution, is excreted entirely and specifically by the kidney, following its injection under the skin or into a vein. In the urine it turns red on the addition of alkali and can be determined quantitatively in a colorimeter by comparing the color of a known dilution of the urine with that of a solution of the dye of known strength. The percentage of this dye recovered in the urine within two hours is used clinically as a measure of the functional capacity of the kidneys. Normally, following the injection of 1 cc. of its solution (6 milligrams of the dye), from 60 to 80 per cent is recovered in the urine within two hours. When the kidneys are diseased smaller amounts are returned, while in advanced renal insufficiency or uremia, no dye at all is recovered. Because of its simplicity this valuable test can be carried out anywhere by any physician.

Similarly, the function of each of the two kidneys may be determined separately by passing small catheters

into the ureters (the tubes leading from the kidneys to the bladder). By comparing the amount of dye from the two sides it is possible to learn whether one or both kidneys are diseased and also the extent of the disease present. This is obviously of great value, particularly in relation to the surgery of the kidney.

The Level of Blood Urea and Creatinine.—In health, urea, a waste substance, and the end-product of protein metabolism, is excreted by the kidney almost as soon as it is formed. The level of the urea in the blood in health is usually about 20 to 30 milligrams in 100 cc. of blood. In serious disease of the kidney it may fail to be excreted, and therefore accumulate rapidly in the blood, often increasing to ten or twenty times normal. It is determined quantitatively in a simple way by subjecting it to the action of urease, a ferment obtained from the soya bean, which converts the urea into ammonia, which in turn can be determined quantitatively by several methods. Recently it has been recognized that the urea in the saliva bears a constant relation to that of the blood; consequently the function of the kidney may be determined by estimating the urea of the saliva and applying the so-called salivary urea index. It must be remembered, however, that the urea, both of the blood and saliva, can be influenced to a certain extent by reducing the amount of protein in the diet. Consequently creatinine, another chemical substance in the blood, is often preferred because it represents an end-product of metabolism which is unaffected by diet. These tests both furnish most reliable evidence of the function of the kidney, and the latter affords the most reliable information available concerning the probable outcome

of the disease, particularly in chronic forms of kidney disease.

The Diluting and Concentrating Capacity of the Kidney.—In addition to these tests, much can be learned concerning the function of the kidneys through determining their ability to dilute and concentrate urine as occasion demands. This can be tested by the dilution test, which consists of administering large quantities of water for one day and restricting food, and by the concentration test, which consists of giving large quantities of solid food for a day and restricting water. The effect of these procedures on the amount and specific gravity of the urine shows how well the kidney is functioning. When dropsy or a tendency to dropsy exists, the kidneys fail to excrete water well, and they can rarely dilute properly. In those forms of nephritis and uremia, unaccompanied by dropsy, the water may be readily passed, but the urine is dilute, and poor in solids. Since the kidney is incapable of excreting solids properly, they accumulate in the blood and tissues and uremia is likely to develop. Knowledge gained from these tests has considerably clarified our understanding of kidney disease.

BLOOD CHEMISTRY—A NEW AND IMPORTANT FIELD IN MEDICINE

The discussion of the urea and creatinine of the blood introduces the subject of blood chemistry, which represents one of the most recent important developments in the field of diagnosis. Whereas formerly in the study of the diseases of the excretory organs, chief emphasis was placed on the changes in the character of the excretion, for instance the urine or bile, the present tendency is toward the study of the blood in order to

ascertain what has accumulated there as the result of the failure of the excreting organ to carry out its function. This change in viewpoint was essential to progress.

Routine Examination of the Blood.—The routine examination of the blood involves as a rule a study of its richness in cells and the concentration of certain of its chemical constituents as compared with the normal. The red and white blood-cells, in a minute but measured quantity of properly diluted blood, are counted under the microscope in a specially constructed chamber. The oxygen-carrying substance of the blood, or the hemoglobin, that is, the red substance contained in the corpuscles, is estimated in a very simple manner. A measured quantity of blood is taken into a tube, properly diluted, and the coloring matter set free by the addition of a small amount of acid. The intensity of the resulting color is contrasted in a special instrument with that accepted as normal. The hemoglobin present in the blood is expressed in terms of relative percentage. A low percentage indicates anemia, either primary or secondary.

The primary is the dreaded pernicious anemia which has certain characteristics whereby it may be identified in the majority of instances. The secondary type of anemia is of frequent occurrence after hemorrhage and in many types of infectious disease. It is also common in diseases accompanied by frequent hemorrhages, such as cancer, ulcer of the stomach or bowel, hemorrhoids and uterine tumors.

Colloids.—During recent years the study of colloidal chemistry has gained increasing momentum. The following relationships may exist between two substances: a simple mixture, the solution of one in the other, or a chemical union in which each loses its properties, more

or less, and its individuality. All such relationships are found in the body, but there is another, the colloidal relation, neither solution nor true chemical union, which applies particularly to animal tissues because of the nature of many of their constituents. Suspensions like milk of magnesia, emulsions like cow's milk and gaseous suspensions like foam represent typical colloidal systems.

When gelatin is boiled in water and allowed to cool, an intimate association is formed which is not solution, since the water has disappeared, and not chemical union, since neither constituent has lost its integrity. In the fluid state the plasma (the fluid portion of the blood) is a solution of more or less intricate chemical bodies, one of the proteins of which is capable of such change that it becomes no longer soluble. It does not then form a true precipitate, but unites with the water of the blood in this peculiar colloid state similar to the union of water and gelatin. This results in a formation of a clot. Substances which have the property of existing in this relationship with fluids are called *colloids*. They include the very complex bodies, the proteins, and are the nucleus of the structure of living tissue.

Although the body is largely composed of water, it is for the most part not water in the usual physical sense, but water bound in this physico-chemical union with colloids. A cell is not a sac containing an ordinary solution, but an intimate colloidal arrangement of very complex chemical bodies in water, together with crystalloids, which is capable of carrying on the chemical activities known as life only so long as the delicate poise of the mechanism is not disturbed.

The importance of colloid chemistry in biology becomes more and more obvious. In the field of diagnosis,

in its wider sense, including the recognition of the progressive changes that lead up to a disease, relatively little benefit has accrued up to the present, but there can be little doubt that colloid chemistry will contribute much. The coagulation of proteins is a colloidal reaction, and the so-called Lichtheim coagulation of the spinal fluid aids in the early diagnosis of tuberculous meningitis. The gold sol reaction of spinal fluid, dependent on the presence of protective colloids as the result of disease, is of value particularly in syphilitic diseases of the central nervous system.

Many methods availing themselves of the separation of substances by means of the unequal rate of passing through a membrane (dialysis) belong here, such as the colorimetric determination of the hydrogen-ion concentration (true acidity) of the blood after dialysis. The rate of sedimentation of the red blood-corpuscles has been claimed to be of special benefit in determining the progress of severe infections. It has been brought in relationship to changes in the relative amounts of the various colloids (the proteins) in the blood. The osmotic power of the blood may aid to distinguish various forms of edema (dropsy). The ingenious test of McClure, who determines the time of disappearance of a small amount of common salt solution injected into the outermost layers of the skin, measures the affinity of a colloid system for water.

It is apparent that no study of chemistry short of complete elucidation of the properties of colloids and the laws governing their activity can ever solve the problem of life and of the essential difference between health and disease. Medicine looks hopefully to this new science.

Inorganic Constituents.—But blood chemistry leads us into many other fields. The study of the inorganic constituents of the blood bears an important relationship to disease. The blood normally contains, among other chemical bodies, sodium, calcium, potassium, phosphorus and chlorine in combinations either among themselves or with the organic constituents of the blood. These have to do with the acid-base equilibrium and the capacity of the plasma to combine with carbon dioxide, to be discussed later. In tetany, a deficiency disease characterized by increased nervous irritability, spasms of the feet and hands, and at times convulsions, the amount of calcium in the blood is decreased, while that of phosphorus is increased. In tetany, due to obstruction of the pylorus (outlet of the stomach) or the first part of the intestine, the chlorine of the blood is usually diminished, the urea increased and the capacity of the blood to combine with carbon dioxide increased.

Blood Sugar and Diabetes.—As already indicated, the continued presence of glucose in the urine is important evidence of the presence of diabetes. But in addition, chemistry has cleared much of the obscurity of our knowledge of diabetes and its complications. The sugar of the blood is now determined quantitatively. A high level of glucose in the blood furnishes evidence of diabetes. On the other hand, a low level of blood sugar with persisting excretion of glucose in the urine, argues in favor of renal glycosuria rather than true diabetes.

In the early stages the diagnosis of a mild form of diabetes may offer considerable difficulty. Here an overloading test is employed. Such a test is designed to overburden or tax the capacity of the organism to metabolize sugar. It is called the sugar-tolerance test, and is one

of considerable importance to the diagnosis. This test is of great value in determining the presence of diabetes in its earliest stages.

Acidosis.—Chemistry also aids us in determining the presence or imminence of the most dreaded complication of diabetes, acidosis, which, in the pre-insulin days, was the most frequent cause of death. Acidosis manifests itself clinically and chemically but the chemical studies yield important quantitative information concerning the probable outcome of the disease and the urgency of the need for treatment. The clinical evidence of acidosis consists of air-hunger or exaggerated breathing, a sweet mawkish odor of acetone on the patient's breath, and often stupor or unconsciousness known as diabetic coma. Chemically, acidosis may be recognized in several ways: (1) the presence of acetone in the patient's breath, (2) the presence of products of incomplete combustion of fats in the urine, especially of diacetic acid which gives a port-wine color on the addition of ferric chlorid solution, and (3) by methods to be discussed presently.

Insulin is a specific in the prevention and in the treatment of diabetic acidosis, but it is a two-edged sword, capable of causing coma, and even convulsions, if improperly handled. In the recognition of this complication, chemistry again comes to our assistance. Insulin shock develops in patients unduly susceptible, or when the dose employed has been excessive. The blood sugar may be found to have reached an excessively low level. Increasing the sugar content of the blood, as by drinking orange juice, or the injection of glucose in any form, usually takes care of this complication.

Maintaining the Reaction of the Blood.—Acidosis

brings us to the subject of the acid-base equilibrium of the body, one of the most interesting chemical matters in the whole realm of medicine. For the normal life and function of each cell of the body a constant environment is essential. In consequence, the volume and composition of the blood must be regulated within very narrow limits. The blood must be maintained practically as a constant. This is particularly true in the matter of its reaction, and the acid-base equilibrium of the body is steadfastly maintained, despite the constant introduction of acids and bases in our food. The slightest variation in the reaction of the blood is likely to prove serious. In fact, in order that life be maintained, it is necessary to keep the blood less alkaline in reaction than ordinary tap water, and less acid than distilled water, since either of these extremes will probably result in death. The mechanism that maintains the acid-base equilibrium is as ingenious as it is effective.

The transfer of carbon dioxide from the tissues to the breath is interfered with in acidosis, since the power of the blood to combine with this gas varies with the acidity or alkalinity of the plasma. The retention of carbon dioxide in the tissues can be measured indirectly by determining the power of the plasma to combine with carbon dioxide experimentally, and directly by measuring the partial tension of carbon dioxide in the breath. Acidosis is accompanied by a reduction in both. The presence of acidosis can also be recognized chemically by the colorimetric comparison of diluted plasma, after the addition of certain indicators, with solutions of known hydrogen-ion concentration, or physicochemically by the gas-chain method. It may also be recognized by the amount of sodium bicarbonate that

must be administered to the patient before the urine is rendered alkaline. Acidosis is always secondary to some other disease, and is commonly encountered in diabetes, in certain forms of nephritis characterized by the retention of phosphoric acid, in the dehydration of the body caused by cholera, or other forms of severe diarrhea (especially in infancy), following the excessive ingestion of salts of ammonia, and in many forms of toxemia.

In contrast to acidosis, is the condition known as alkalosis, wherein the blood becomes overly alkaline. This is a striking finding in some cases of high intestinal obstruction in which a peculiar and almost characteristic combination of chemical findings is present.

The Rate of Metabolism and the Cost of Work.— Another fixed function of the body in health is the rate at which metabolism proceeds. This may be accurately determined quantitatively under basal conditions, that is, with the patient at rest and at least twelve hours after eating. While in reality, the heat production in the body is the measure of metabolism, actually it is calculated from the oxygen consumed and the carbon dioxide expired. This gas analysis is, of course, a chemical problem.

It has been predetermined that, under these basal conditions, the oxidation in the body of an adult man shall proceed at such a rate as to liberate 39 calories an hour for each square meter of skin surface. The rate of metabolism is controlled by the chemical principles in the blood, prepared by the glands of internal secretion, more especially by the thyroid gland, which act on metabolism as a "pair of bellows on a fire." Therefore, it is not surprising that in the diseases of this gland we may have striking variation from this fixed normal.

The quantitative determination of such changes is of the utmost importance in diagnosis. In overfunction of the thyroid gland, whether associated with diffuse disease of the gland (exophthalmic goiter) or with tumor of the gland (toxic adenoma), there may be a striking increase in the rate of metabolism, sometimes a 100 per cent increase. In certain other diseases, myxedema and cretinism, the basal metabolism is markedly lowered, sometimes 40 per cent below normal. In disease of the pituitary gland, also, and in certain other conditions as yet little understood, a lowered rate may also be encountered.

Bodily activity obviously requires the liberation and expenditure of energy and this is made possible through the metabolism of food. The work accomplished in the steamship is readily standardized and the cost of this work in coal readily computed. Similarly, the cost of bodily work in calories can be computed. In some diseases, work costs even as much as double what it normally should. This is especially striking in exophthalmic goiter, a disease familiar to all by reason of the characteristic "pop eyes." This "high cost of work" is responsible for the ravenous appetite and the marked loss of weight which are also characteristic of the disease.

USING THE BODY FOR TESTS

Hormone Deficiencies.—The body is used at times in place of a test-tube for carrying out chemical tests. Instead of determining changes in the color of solutions or the development of precipitates, we may recognize the presence or absence of certain chemicals, natural or foreign to the body, by specific changes in the body or its function. Such a procedure is known as a therapeutic

test and is utilized at times in establishing a diagnosis. A chemical substance may be introduced into the body, to replace one that is thought to be missing (thereby re-establishing normal function) to set up certain changes in the body fluids whereby the disease can be more readily recognized chemically, or to effect changes in symptoms which are known to respond specifically to certain chemicals.

At times the body may appear to be lacking in some internal secretion or hormone. If the replacement of this by a corresponding glandular product results in cure, the diagnosis is established with more or less certainty. Only recently, a striking example of this came under our notice. A patient, a middle-aged woman, came from Cuba, because of her inability to regain health, despite visits to innumerable doctors. She complained of tiredness, retardation in thought and action and intolerance to cold. Her complexion was pale and pasty, the skin of the face infiltrated and the wrinkles obliterated. Marked anemia and a lowered rate of metabolism were found on laboratory investigation. Underfunction of the thyroid had been considered by her home physician, a Cuban doctor of international fame, and treatment had been instituted many years ago but discontinued because of the discomfort it occasioned. This original diagnosis was ignored by all subsequent doctors because the patient had received specific treatment with thyroid and it was accounted a failure. Yet after five years, when this patient was placed on the same treatment and it was cautiously continued for some weeks, her recovery was striking and complete. The diagnosis was strongly suggested by the low basal

metabolic rate, and was confirmed by the response to a specific form of treatment.

This specific replacement of products of the ductless glands is of value in other diseases of the glands of internal secretion. Thus, in Addison's disease, due to deficiency of the suprarenal glands, in which the patient becomes darkly pigmented, loses weight and suffers from fainting and dizziness, loss of appetite, nausea and vomiting, a doubtful diagnosis may be converted at times into a certainty through the administration of the glandular product, epinephrine, which the patient has failed to produce. Similarly in diabetes insipidus, a disease of the pituitary gland characterized by great thirst, the drinking of enormous quantities of water, and the voiding of comparable amounts of urine, the diagnosis, if in doubt, may often be established by the hypodermic administration of the extract of the posterior lobe of the pituitary gland, which in this disease checks thirst and reduces the output of urine. The same principle holds true in tetany, due to destruction or removal of the parathyroid glands. Here the calcium content of the blood is low as the result of malfunction of the parathyroids. The extract of this gland re-establishes the normal level of blood calcium and abolishes the symptoms.

Other Tests.—Therapeutic tests are used in still other types of disease. In the laboratory, syphilis is commonly diagnosed by means of the Wassermann test. In the beginning of the treatment, the administration of antisyphilitic drugs often results in an exaggeration of this Wassermann reaction. Daily, advantage is taken of this

fact to obtain such evidence of syphilis when the clinical findings and other laboratory data leave room for doubt. This is known as a provocative test for syphilis.

It is obvious that this principle might be adopted quite generally in certain diseases that respond only to certain forms of treatment, but generally speaking, it is not sufficiently direct and consumes too much time. However, in angina pectoris, a disease characterized by breast pang, it may prove of great value. This disease is often confused with other diseases which, however, do not yield promptly to the influence of nitrites as angina pectoris does in the majority of instances. In suspicious cases, therefore, the immediate relief which comes from inhaling a few whiffs of amyl nitrite constitutes important diagnostic evidence of the presence of angina pectoris in the majority of instances.

The Behavior of Dyes in the Body.—Dyes may render signal service when used as test substances. Reference has been made already to the use of phenolsulphonephthalein as a test of kidney function. It answers our present need admirably since it serves to introduce the subject of the relation of the chemical constitution of a substance to its behavior in the body. Much has been written on the subject of chemical constitution and the pharmacological action of drugs, but less attention has been paid to the relation of chemical constitution and the excretion of substances. The close chemical relationship of the phthaleins used in the form of their sodium salts in the diagnosis of diseases of the kidney, liver and gall-bladder, is shown in the following list of the substances used and their chemical formulas:

The red salt of the original phenolphthalein, used for many years by chemists as an indicator of acidity or alkalinity, is

$$NaO_2C.C_6H_4C(C_6H_4ONa)(:C_6H_4:O)$$

Sodium phenolsulphonephthalein, used in testing the functioning of the kidneys, is

$$NaO_3S.C_6H_4C(C_6H_4OH)(:C_6H_4:O)$$

Sodium phenoltetrachloro- and -tetrabromo-phthaleins, used as tests of liver function, are

$$NaO_2C.C_6Cl_4C(C_6H_4ONa)(:C_6H_4O)$$
$$NaO_2C.C_6Br_4C(C_6H_4ONa)(:C_6H_4:O)$$

Sodium tetraiodo- and -tetrabromo-phenolphthaleins, used in preparing the gall-bladder for study with the Roentgen ray, are

$$NaO_2C.C_6H_4C(C_6H_2I_2ONa)(:C_6H_2I_2:O)$$
$$NaO_2C.C_6H_4C(C_6H_2Br_2ONa)(:C_6H_2Br_2:O)$$

The list evidently represents a beautiful illustration of the resources of creative chemistry through variations to meet the varied needs of medicine.

Phenoltetrachlorophthalein is excreted specifically and only in the bile in health, and the amount of excretion is an index of the function of the liver. Owing to difficulties in collecting bile, the retention of the dye in the blood after its ingestion has been determined, in place of the rate of its excretion. This is an excellent index to the functional capacity of the liver, especially in disease. The use of compounds that render the gall-bladder opaque to the Roentgen ray is more recent. The

discoverer of the method states that he can recognize disease of the gall-bladder in more than 95 per cent of cases, while another authority found that the diagnosis made in this way was confirmed at operation in 76 per cent of a large number of cases.

How Infectious Diseases Bring about Peculiar Changes in the Blood: Serological Tests Used in Infectious Diseases.—The Wassermann test, named after the man who originated it, belongs to a group of serological tests. This is a field of diagnosis which represents a development of the last few decades. All serological tests, though crude from the viewpoint of the chemist, yield reactions specific to a given disease and hence are of the greatest value to the physician in the making of a diagnosis. Such tests depend on so-called immunological reactions of blood constituents produced by disease, the nature of which, as far as it is at present understood, is discussed at length in another article on immunity (see p. 559). At this place we shall limit ourselves to a consideration of some of the applications of these studies specifically to diagnosis.

In the first place, we have the Widal test for typhoid. This depends upon the fact that the diluted blood serum of a patient suffering from typhoid when added to a dilute suspension of typhoid bacteria causes the coalescence (*agglutination*) of the bacteria as observed under a microscope. Although the reaction rarely appears before the tenth day of the disease, it is an important specific aid in the diagnosis of typhoid fever. The reaction evidently is the result of the production in the blood by the disease of a specific chemical principle able to bring about the agglutination of typhoid bacteria. The same principle is present in patients who

have had typhoid previously or who have been recently vaccinated against the disease. Agglutination methods are also used in the diagnosis of several other diseases, such as paratyphoid fever and meningitis, and in differentiating the various forms of pneumonia.

It has been found in man that the blood of one individual may be antagonistic to the blood of another. All blood is found to fall into four groups which show more or less mutual antagonism. This antagonism is manifested by agglutination of the red cells when an incompatible blood is added to it. Which is the agglutinator and which the agglutinated varies with the groups to which the two samples of blood belong. The agglutination is brought about by certain factors which render invading blood sensitive to agglutination. The particular factors, specific for blood of certain other groups, are said to be inherited according to Mendel's law. This incompatibility of blood accounts for the bad effects that sometimes used to follow transfusion, before precautions were taken to see that the donor and donee were in the same group.

Other and perhaps similar chemical principles produced in the blood by certain other diseases have the power to *precipitate* specific foreign proteins characteristic of the invading bacteria. Such precipitation tests made with the serum of a patient on dilute bacterial suspensions are used in the diagnosis of poliomyelitis, an infection of the spinal cord especially menacing to children, in the differentiation of types of organisms in pneumonia and in the diagnosis of echinococcus (a tapeworm) disease, characterized by multiple cysts, which is very prevalent in sheep-raising countries like New Zealand.

A somewhat more complex serum test is used as a specific test for syphilis, the famous Wassermann test, which has proved extraordinarily useful in diagnosis. It has now become practically a routine test in all hospitals because the disease is often masked except for rather obscure symptoms.

Another most important application of serological testing is used in diphtheria, not by withdrawal of any blood but on the body itself of the patient, the so-called Schick test. This depends on a reaction between the diphtheria toxin, a poison produced by the diphtheria germs, and diphtheria antitoxin, a defensive chemical principle produced in the blood to neutralize the poison. In the Schick test, diphtheria toxin, in proper amount and dilution, is injected into the outer layer of the skin; in the absence of an immune substance (antitoxin) in the blood and tissues of the patient, it will give rise to a well-defined inflammation, while in the presence of an antibody, no such inflammation takes place. In this way, it is possible to predict who is or is not protected, and which of a group of people are likely to contract diphtheria when they are exposed. The test is of enormous value in protecting children against diphtheria. In some cities the test is becoming about as prevalent as vaccination against small-pox.

A somewhat similar test has been developed by Dick and Dick during the last two years, to show whether or not a person is susceptible to scarlet fever. This test is certainly promising, but sufficient time has not elapsed to permit the establishment of this test on the same firm basis as the Schick test.

Future Progress.—It was not intended that every chemical process related to disease and the detection of

disease should be explained here. Space will not permit of more than a general presentation of the subject with sufficient illustrations to indicate how closely chemistry touches the problems of life and disease. Indeed the general application of chemistry to medicine is too recent and the problem too vast for elucidation either now or in our time. Pathology, the science of morbid processes, has for centuries been confined to the study of structural change in diseased tissue. Chemistry has had to correct a long tradition before it could be admitted to its proper place in pathology. In Boerhaeve's days, two centuries ago, the chemists were considered "whimsical dabblers." Now chemists interested in the different phases of their science are working in almost every field of medicine. So many of the manifestations of disease are being interpreted in terms of chemistry that the student of medicine is constantly driven to delve deeper into the fellow science. More chemists are finding in pathology and medicine a fascinating and useful field of research; and still more are needed. We are beginning to learn that all evidence of disease is not visible under the microscope and that the beginnings of all disease, and the important manifestations of many diseases throughout their course, may be entirely chemical in nature.

Medicine advances steadily, not by spectacular discoveries, but by adding stone to stone. A life is saved today not so much by the genius of one man as by the unremitting efforts of generations of scientists, and often by the application of discoveries in pure science. Science provides the key to the problems of life and disease, and the laws of the universe find a common meeting-ground in Medicine.

Chapter VIII—Article 2

GENERAL ANESTHESIA

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Professor Luckhardt has done all of his important work at the University of Chicago, with which he has been associated since 1908. He is the famous discoverer of the use of ethylene gas in place of ether, chloroform or laughing gas, in producing general anesthesia for operative purposes. Ethylene has certain advantages, described in the present article, which are leading to its use in ever wider circles. Dr. Luckhardt is also doing authoritative work on the hormones of the glands of internal secretion and on gastro-intestinal secretion.

There is no single discovery in medicine which has been more conducive to the progress of medical sciences as a whole than the discovery of general anesthesia. Little wonder, then, that the greatest experimental physiologist of all times, Claude Bernard, stressed its importance in glowing terms. Admitting that, sometimes, it has modified the results of experimental research so that the interpretations based on them were physiologically unsound, it is nevertheless a fact that our general knowledge of the mode of action of the individual organs and the body, as a whole, in health and disease, is due in the greatest measure to the discovery of general anesthesia. Without it physiology, biochemistry, pharmacology, pathology and bacteriology would have remained in the main barren speculative "disciplines" where the incorrect guesses of those having current esteem would have weighed heavily in the balance against the more fortunate but lucky and possibly

correct guesses of those ranking lower in their colleagues' estimation. Rival claims could obviously not be examined critically; for data could not possibly be collected to decide for or against a given hypothesis or tenet because of the torture which would prevent all but a very few from investigating the problem. And finally, without the data obtained from animals while under, or those which have been operated upon under a general anesthetic, further problems leading slowly but surely to the answer of the riddle of health and disease—of life itself—could not even suggest themselves to the investigator.

Aside from these considerations, the fact remains that three very important general anesthetics were introduced into the practice of human surgery quite some time before the general experimental biological (medical) sciences developed as a result of their introduction. Besides making surgery an art where the operator could more leisurely and with better judgment make such repairs, extirpations, and alterations as seemed indicated whilst the patient was suitably relaxed and unconscious of what was being done, an enormous amount of suffering, amounting often to torture, was forever spared future generations.

Removed from the actual sight and noise of the gruesome operating rooms of preceding centuries, we can only guess what sick humanity must have endured when subjected to surgical intervention. Living in a less prosaic age and struck by the contrast of the old régime and the one which followed the general introduction of ether anesthesia, Warren writes:

"A new era has opened to the operating surgeon! His visitations on the most delicate parts are performed,

not only without the agonizing screams he has been accustomed to hear, but sometimes with a state of perfect insensibility, and occasionally even with the expression of pleasure on the part of the patient. Who could have imagined that drawing the knife over the delicate skin of the face might produce a sensation of unmixed delight! That the turning and twisting of instruments in the most sensitive bladder might be accompanied by a beautiful dream! That the contorting of anchylosed joints should co-exist with a celestial vision! If Ambrose Paré, and Louis, and Dessault, and Cheselden, and Hunter, and Cooper could see what our eyes daily witness, how would they long to come among us, and perform their exploits once more! And with what fresh vigor does the living surgeon, who is ready to resign the scalpel, grasp it, and wish again to go through his career under the new auspices!"

But general anesthesia with its attendant loss of consciousness and general loss of sensation accomplished even more. Not only did the anesthetic state provide a condition which made the daily task of the surgeon technically easier and personally more agreeable, not only was the patient spared untold misery and anguish, but for a variety of reasons the patient's recovery from any given operation was greatly increased. This result, forgotten by the present generation, was noted early by the surgeons and properly evaluated. In a review of several articles and books on surgical anesthesia an early author writes:

"According to these tables the following results were obtained: Mr. Philips records 1,369 cases of amputation of the thigh, leg and arm; the mortality was about 37 per cent. In 242 cases reported from the Glasgow

Hospital, the mortality was about 47 per cent. In 484 cases reported from the Parisian hospitals, the mortality was about 49 per cent; while in 302 amputations of the same class, performed under etherization, the mortality was 23 per cent. . . . We ask any candid and unprejudiced surgeon to consider this matter attentively, and then ask himself the question, whether he can reconcile it to his conscience to withhold agents which would diminish the mortality resulting from surgical operations?"

Early History.—Whereas the discovery of the general anesthetics is in the main an empirical one it nevertheless rests on a very fundamental discovery, namely, on the discovery of oxygen by the chemists. Following the discovery of this gaseous element and its importance in living processes, general interest in other gaseous elements was aroused. Thomas Beddoés (1760-1808), impressed with the importance of oxygen, founded a Pneumatic Institute in England for the treatment of diseases by the inhalation of gases. Humphrey Davy served as an assistant to Beddoés in this "Institute." Among other gases, Davy tested the effects of nitrous oxide or "laughing gas" (N_2O) on himself, and was so impressed with its possibilities in surgical maneuvers that he wrote:

"As nitrous oxide in its extensive operation appears capable of destroying physical pain, it may probably be used to advantage during surgical operations in which no great effusion of blood takes place."

Ether.—As a result volatile substances of various types were soon tried, and among them ether. Whereas the latter was used particularly in the treatment of respiratory diseases, the exhilarating effects following

the inhalation of gaseous ether vapor did not escape the attention of those experimenting with it. That such persons who were under its exhilarating influence suffered no pain from otherwise painful bruises and injuries could not escape the attention of the more thoughtful experimenters. Among those who used ether for so-called "ether frolics" in this country was Dr. Crawford W. Long of Jefferson, Georgia.[1] As a result he was tempted to try its use in surgical operations. This he first did on March 30, 1842, the patient being a Mr. James Venable. He removed another tumor from Mr. James Venable on June 6, 1842[2]. Finding it successful in practice he continued to use it in his private practice in minor surgical work in the ensuing years. In one authentic instance he used it by inhalation to destroy the pain sense prior to the extraction of a tooth. In another instance he, with true scientific spirit, caused a young negro boy to inhale its fumes prior to the amputation of one of his fingers, and then amputated another without its use to convince himself of the pain deadening effect following its inhalation. There can be no question, therefore, that Dr. C. W. Long (1815-

[1]A letter of Dr. Crawford W. Long to Mr. R. H. Goodman reads as follows

Dear Bob:—

I am under the necessity of troubling you a little. I am entirely out of ether and wish some by tomorrow night if it is possible to receive it by that time. We have some girls in Jefferson who are anxious to see it taken. Your friend,

C. W. Long.

[2]The bill rendered for these services reads as follows:

James Venable

1842	To Dr. C. W. Long Dr.	cts.
January 28th	Sulphuric Ether	.25
March 30	Ether & Exsecting Tumour	2.00
May 13	Sulphuric Ether	.25
June 6	Exsecting Tumour	2.00

1878) was the first person to use ether as a general anesthetic agent. Some two years later (1844), as a result of Humphrey Davy's experience with it, Horace Wells had a tooth extracted from himself whilst under the influence of nitrous oxide gas administered to him by Gardner Q. Colton. He suffered no pain whatever. Attempting to demonstrate the efficiency of nitrous oxide at the Massachusetts General Hospital, Wells unfortunately failed, the failure being followed by the derision of those about him, who called him an imposter. Wm. T. G. Maston witnessed this unsuccessful demonstration, and on consultation with Dr. C. J. Jackson (Boston) was induced to experiment with the possibly anesthetic action of inhaled ether fumes. As a result of experimentation on animals and himself he approached John C. Warren of the Massachusetts General Hospital, who states that "calling on me, he stated that he had possession of a means of accomplishing this object, that he had made trials of its efficacy in the extraction of teeth, and that he wished me to test its power in surgical operations. The article used for this purpose not being mentioned, I supposed it was not proper for me to demand what it was, but I did think it necessary, before taking the responsibility of using it, or sanctioning its use, to ascertain whether a trial could be made without any apprehension of danger. Having satisfied myself on this point by various questions, I agreed to give Dr. M. the desired opportunity as soon as it should be in my power."

Because of the successful outcome of this demonstration (Warren operating on a patient etherized by Morton), ether anesthesia was soon generally used both in this country and in England.

Chloroform.—The practically minded scientific world of the time became enthused in research to discover other and even better anesthetic agents. Two men particularly should be mentioned in this search, Dr. James Y. Simpson and Thomas Nunnely. At a chemist's suggestion Simpson with his associates soon discovered chloroform, a powerful anesthetic, and introduced it into surgical practice. Nunnely worked from our present viewpoint on a rather bizarre and dangerous list of substances and introduced no practical and relatively harmless agent.

Examination into the sudden deaths occurring quite some days after the administration of chloroform anesthesia revealed the fact that death was due to what has been since termed a delayed chloroform poisoning characterized chiefly by destructive and fatty changes in the various organs of the body, notably the liver. In spite of its several distinct advantages over ether and nitrous oxide, the insidious and invariably fatal after-poisoning which at any time might possibly follow its administration markedly diminished its widespread use, with the result that chloroform as a general anesthetic agent is used in this country only exceptionally compared with ether and nitrous oxide. It is only fair to state, however, that in Europe chloroform is more commonly used than ether, and that nitrous oxide-oxygen anesthesia is hardly understood and, by and large, never used.

Laughing Gas.—The uncertain anesthetic value of nitrous oxide (laughing gas) administered with air and the more powerful action of both ether and chloroform would have relegated the former to an unjust oblivion had not the late E. Willys Andrews of Chicago demon-

strated that nitrous oxide could be used most successfully in combination with oxygen instead of air. This rejuvenation of the use of nitrous oxide with oxygen sufficed to restore this general anesthetic to a field of usefulness which its physiological properties merit. As a consequence the literature was soon deluged with papers on its successful administration for the benefit of suffering humanity. Most of the writers on this form of anesthesia have themselves contributed very little except that the continuous confirmation by a host of anesthetists not only amply justified its use but also established its superiority in a select percentage of cases in which ether and chloroform were not required, but were perhaps even contra-indicated.

With respect to the practice in this country it might be said that there existed but two general anesthetics, namely, ether and nitrous oxide, or laughing gas, until the spring of 1923.

Ethylene and Oxygen.—Interest in general anesthesia as a whole was renewed when Luckhardt and Carter published their preliminary accounts, not only on the physiological effects of ethylene gas on the usual laboratory animals and normal men, but, within a month, on the successful use of this gas (in proper concentration mixed with oxygen) as an anesthetic agent in the surgical clinic. In a rather thorough clinical study on about 900 patients requiring surgical intervention no immediate dangerous or untoward effects were noted. This new form of anesthesia was therefore committed to the surgical world for further clinical trial. A number of favorable reports were soon issued regarding its use in various medical fields—genito-urinary surgery, gynecology and obstetrics, pediatrics, and dentistry. As a

result its use became rather widespread within a comparatively short time.

Ethylene-oxygen anesthesia owes its popularity to a number of desirable properties, all of which are possessed by no one of the other available anesthetic agents. Ethylene causes an anesthetic state almost as profound as ether and more profound than nitrous oxide. Because of this fact the relaxation of the patient is more complete than with nitrous oxide, allowing more accurate and careful surgical technic. Since the relaxation is almost as profound as when ether is used, but without the disagreeable and prolonged subsequent ether sickness, ethylene is preferred to ether when the *utmost* relaxation is not required. Patients have been anesthetized for as long as 5.5 hours, with a maximum period of nausea and vomiting of one hour directly attributable to the anesthetic. In spite of the profound anesthetic state effected by ethylene administered in combination with oxygen, patients return to the conscious state within 2 to 3 minutes usually following removal of the mask irrespective of the duration of the surgical anesthetic state. Of a gaseous nature and given in combination with oxygen as is nitrous oxide, ethylene, like nitrous oxide, is non-irritant to the respiratory passages, and is therefore not contra-indicated, as would be ether, in patients who at the time of operation suffer from an acute upper respiratory infection, acute bronchitis, or pulmonary tuberculosis; ether because of its irritant properties may predispose patients having these respiratory infections to a fatal post-operative pneumonia. Since any given surgical anesthetic state can be induced with more oxygen than is possible under nitrous oxide, general asphyxia of the cells is avoided, with its unfavorable consequences.

Incidentally, the surgical and anesthetic sleep under ethylene is tranquil and more nearly like the normal sleep than is the case under any other anesthetic agent. Salivation and perspiration are less marked under nitrous oxide than under ether, and least under ethylene. Not only are the body fluids conserved as a result (for the patient may not be allowed to take fluids for some time after recovery from the anesthetic) but the absence of chilling resulting from the perspiration minimizes the danger of a post-operative pneumonia. Certainly, the induction to the anesthetic state is more pleasant with nitrous oxide or ethylene than it is with ether. Most people find early induction with nitrous oxide even more pleasant than ethylene, for the reason that the odor of ethylene is disagreeable to some. Since, however, the olfactory sense is anesthetized efficiently and promptly by the first few inhalations of the gas, this feature is of no serious consequence, particularly since it can be circumvented technically, if desired, in a very simple manner.

Acetylene.—About the same time that ethylene anesthesia was developed in this country, Gauss and Wieland reported the anesthetic properties of acetylene (sold as "Narcylen"). As might be expected from its chemical composition (C_2H_2), this agent has physiological properties similar to ethylene (C_2H_4). The author not only saw it used in the clinic of Professor Gauss at the University of Würzburg, but was anesthetized there by him (1925), so that he might be able to compare its action with ethylene which he has taken several hundred times.

Acetylene is a more powerful agent apparently than ethylene, since it can be given usually with a higher percentage of oxygen. But it was difficult to come to definite conclusions on this point, since most patients

personally seen had been rather heavily premedicated with pantopan and scopolamine. Furthermore, in abdominal operations, local infiltrations of the abdominal wall with cocaine and epinephrine (adrenaline) was commonly practiced. This made evaluation of the potency of the agent itself rather difficult.

The anesthesia was satisfactory in every particular to the surgeon, but it seemed to be present with less muscular relaxation. A generalized spastic condition coming on without warning was due perhaps to faulty administration. Apparently, however, spasms of the jaws and pharyngeal region interfering with respiration and suddenly precipitating an asphyxial state were of frequent occurrence. Such an alarming status has never been observed to occur under ethylene anesthesia. On recovery from an otherwise pleasant sleep, the author's jaws felt cramped for quite some time, a phenomenon closely related to the one just described. The acrid (almost acid) taste and smell make the induction period less pleasant than with ethylene. However, the experience was not at all unpleasant. Certainly, both patients and the author salivated more profusely than under ethylene, and the period of recovery was more prolonged and less free from a tinge of nausea. By and large, the subjective effects of acetylene and ethylene are qualitatively almost identical.

Nevertheless, acetylene has not been generally employed in Europe, and no extensive clinical reports from various quarters have been issued as yet. In this respect its use clinically rests, at present, on a weaker basis. Goldman and Goldman have used it quite successfully in this country in the extraction of teeth. Professor Gauss in a recent letter states that progress in acetylene

has been made and that the results will be published presently.

At present there are then three well established general anesthetics in quite general use throughout the civilized world (ether, chloroform and nitrous oxide).* Experience gathered in the ensuing years will determine whether ethylene, commended alike by surgeon, anesthetist and patient during the past four years, will take equal rank with those already firmly established.

Benefits of General Anesthesia.—No one can fathom or express the untold misery which the discovery of general anesthesia has forever abolished from this world. It has alleviated suffering and prolonged life of man and animal in three distinct ways. First of all, general anesthesia has made it possible for the modern surgeon to operate with certainty of preserving life, when before its discovery the patient was left in his torture until death itself relieved him of his suffering. Secondly, general anesthesia forevermore stopped the groans, screams and hideous cries which must have sounded like the usual conceptions of the orthodox inferno. Such a situation prohibited cool judgment as to what to do, and, even if it did not, certainly prevented the technical and mechanical execution based on reflection and surgical judgment. Finally, surgical anesthesia was the portal of entry to that large and fruitful field of experimental biology, inasmuch as it permitted research on the lower animals operated on under its influence or prepared under its influence for the study of biological problems. No

*Ethyl chloride has not been discussed because its use as a general anesthetic for prolonged operations has never been seriously advocated. The interested reader will find a chapter on ethyl chloride in "Anesthesia" by Gwathmey MacMillan Co., 2nd edition, 1924, p. 280.

serious minded man or woman has ever questioned the value of rational empiricism. However, the greater part of both our knowledge concerning living processes in health and in disease and prevention of disease itself has been obtained from the lower animals, studied under the influence of anesthetics or prepared for study while under their influence for subsequent painless experimentation. The accepted technic of the surgeon used daily in every clinic of the world was conceived because it could be tested out and perfected by experimental work on the higher mammals while in the anesthetic state.

Chemical Purity of Anesthetics Vital.—The purity of any one of the general anesthetics obviously is essential to its safe administration. Even small proportions of toxic substances present as impurities inhaled continuously would lead to disastrous and even fatal results. For instance, chloroform ($CHCl_3$) exposed to the air and sunlight under certain conditions is oxidized to phosgene ($COCl_2$), the deadly poison of chemical warfare in the Great War! In the manufacture of ethylene carbon monoxide, the deadly poison of the charcoal stove and of the automotive engine, is liable to be produced as a by-product, and some early fatalities in the use of carelessly prepared ethylene were found to be due to the contaminating carbon monoxide and not at all to the anesthetic (see p. 395). Anesthetics are prepared therefore under the critical control of chemists and the finished preparations must meet the most searching tests of purity prescribed for ether, chloroform and laughing gas for surgical use by the U. S. Pharmacopeia and for ethylene by the Council on Pharmacy and Chemistry of the American Medical Association. The use of any preparation that does not meet these search-

ing chemical requirements would amount to criminal carelessness! Ordinary brands, not especially prepared for surgical use, must under no conditions be used. Furthermore, ether and chloroform are susceptible to decomposition when exposed to air and sunlight. For that reason they are usually taken from small sealed containers, freshly opened for use; any residue in the container is discarded and not used for later operative work in the best practice. The many thousands of operations carried out safely with these precautions show how carefully the safety of the patient is guarded by chemistry in this field.

Modern surgery rests on anesthesia and asepsis. Two tablets over the entrance to the surgical division of the University of Chicago Clinics will forever commemorate this indebtedness.

Anesthetists.—In conclusion, I should like to pay a tribute to our anesthetists. This responsible profession has never received from the medical profession and intelligent lay public the recognition which it richly deserves. After all, the life of the patient subjected to surgical intervention is directly in the hands of the anesthetist, who must not only choose the anesthetic best suited to the patient and the requirements of the surgeon but must be technically so proficient in its administration that the patient will suffer the minimum damages as a result of its use. Unfortunately, the tendency to credit the surgeon for the successful outcome of any operation is too common. Much of his success, and indirectly all of it, is due to the art and science of his anesthetist. Why not give credit to whom it belongs instead of crediting the anesthetist, as is usually

done, with all of the untoward results? After all, most of the credit for the relief of distress, pain, torture and the unshocked recovery in human and animal surgery is due to anesthesia and the intelligent administration of the anesthetic agent.

Chapter VIII—Article 3

LOCAL ANESTHESIA

ERNEST H. VOLWILER, Ph.D.

Chief Chemist, Abbott Laboratories, Chicago

Since 1920 Dr. Volwiler has been the chief chemist of the Abbott Laboratories, one of the great American houses manufacturing medicinal products, which devote large sums in their research laboratories to the discovery and introduction of new remedies. Dr. Volwiler was associated with Professor Roger Adams of University of Illinois in the discovery of the water-insoluble local anesthetic, butesin, and of butyn, a local anesthetic which, for use on mucous membranes, such as the surface of the eye, is said to be superior to procaine and other local anesthetics.

It has been truly said that pain is a useful servant but a harsh master. Though necessary in protecting the body from harm or destruction, pain is nevertheless a useless accompaniment in incurable disease and in surgery, leading to discomfort, shock, and even death.

The earliest historical records indicate that many attempts, more vigorous than successful, were made to alleviate pain in surgery. They were essentially crude efforts to accomplish local anesthesia. Freezing or markedly cooling the part to be operated upon by means of ice or exposure was practiced, and this method in a refined form is still employed for slight superficial operations, volatile liquids such as ethyl chloride and ether being sprayed on the area until it is frozen. Another method consisted of the use of compression, which benumbed the nerve trunk and produced some anesthesia of a limb; but the method was destructive and the pres-

sure produced almost as much pain as that which it was designed to relieve. Chinese, Egyptian, Greek, Roman, Indian, and African medical men were acquainted with the narcotic properties of the juices of certain plants, such as the poppy, henbane, mandrake leaves and roots, Indian hemp, and others; the patient was given a drink of one of these juices, or of alcohol, to put him into a stupor during the operation.

Modern Anesthesia.—These early methods were crude, and woefully unsatisfactory. It is an interesting fact that less than a century has elapsed since surgery first had the benefit of dependable narcotics. (The term "narcotic," as here used, refers to substances which will produce sleep and the word here has no relation to habit-forming properties.) The anesthetic properties of nitrous oxide (laughing gas), ether and chloroform, did not receive general recognition until well along in the nineteenth century, and modern surgery owes its development to their introduction. Ether had been known for centuries; nitrous oxide was discovered by Priestley in 1776, and chloroform by Guthrie in 1831; each of them had been toyed with because of their recognized power of producing unconsciousness, yet it was a long time before the final step was taken and the boon of efficient anesthesia made available to mankind. Strangely enough, all three of these substances were seriously introduced as aids in surgery between the years of 1844 and 1847.

In spite of the present well-nigh universal use of ether, chloroform, and nitrous oxide, together with some other compounds of later development, certain drawbacks to their use could not escape recognition. After an operation under general anesthesia by these

agents, the patient frequently experiences nausea, an acidosis is set up, liver damage may result, and the pulmonary irritation produced by the general anesthetic may contribute to post-operative pneumonia. While general anesthesia will probably continue to be generally employed for certain types of operations, its use in many fields has been largely curtailed by the introduction of modern local anesthesia, whereby many of the objections of the former method are avoided or minimized. For many purposes, too, the administration of a local anesthetic, in general surgery and in dentistry, is more convenient and expeditious.

The Use of Local Anesthetics.—This mode of produsing insensibility to pain is designed to affect only the area of operation, leaving the important normal body processes unimpaired. The so-called terminal or infiltration anesthesia is accomplished by injecting an aqueous solution of the anesthetic directly into the region where the anesthesia is to be produced, whereby the nerve terminals are paralyzed. In regional anesthesia, or nerve blocking, on the other hand, an injection is made in the neighborhood of a nerve trunk, which makes insensible the region supplied by that nerve. In addition, topical anesthesia is employed in the eye, ear, nose, throat, urethra, and on broken areas of skin; in such cases, the anesthetic is not injected, but the solution or the solid itself is applied directly to the area to be anesthetized. Both water-soluble and water-insoluble anesthetics are employed for such purposes.

Where the local anesthetic needed to be injected, no progress could be made until a method of application was made available. This was accomplished by the introduction of the hollow hypodermic needle by

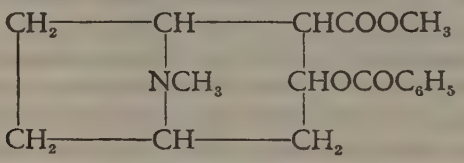
Alexander Wood of Edinburgh, in 1853. Even then, there was no local anesthetic available which could be injected.

Discovery of Cocaine.—At the time of the Spanish conquest of Peru, in the first half of the sixteenth century, it was noted by the followers of Francisco Pizarro, that the Indians, to avoid fatigue and appease hunger while carrying their burdens in that mountainous country, were in the habit of chewing the leaves of a certain plant, coca erythroxylon. Some of these plants were later cultivated in Europe, where it was found, as the Peruvians already knew, that continued use leads to mental and physical degeneration.

It was not until 1855 that Gädeke succeeded in isolating the alkaloid cocaine from the leaves of the coca plant; and in 1860 Niemann, a student of Wöhler in Germany, obtained it in crystalline form and studied its properties. Although the anesthetic action when coca leaves are chewed, or when cocaine is placed upon the tongue, was recognized by Europeans as early as 1855, and some sporadic suggestions as to its possible use in surgery were made, the real application of the product did not develop until Koller of Vienna, and later of New York, used it in surgery of the eye in 1884. Effective local anesthesia dates from this event.

Invention of Better Local Anesthetics by Synthetic Chemistry.—Great enthusiasm over the discovery of this new and invaluable aid in surgery quickly led to careless use and disaster. It was soon found that cocaine is quite toxic, contributing to occasional deaths, and possesses the serious fault of leading to habit formation. In addition, the solutions are unstable.

Nature was apparently unable to provide any product superior to cocaine; but, fortunately, at that time, synthetic chemistry was just getting on its feet, and here was a challenge to the organic chemist. The difficult task needed to be performed, and it was undertaken by some of the most brilliant chemists of that time. A superior substitute was required, and this necessitated a knowledge of the structure of cocaine. Repeated attacks on the problem by Merling, Einhorn, Ladenburg, Willstätter and others, solved the very complex problem. It was found that cocaine has the structure:



COCAINE

Inspired by the hope that not all the parts of this complex molecule are necessary to produce anesthesia, the chemists set about dissecting it to find which portions are necessary. There were found to be three important parts of the cocaine molecule: on the left side of the formula shown above is a group of atoms, or a ring, as it is called, which is common with a series of alkaloids, such as nicotine; but it is not essential to produce local anesthesia. The right-hand ring, which also occurs in other alkaloids, such as coniine obtained from the poison hemlock which accomplished the death of Socrates, plays somewhat of a rôle in the anesthetic effect produced by cocaine. Finally, the —$OCOC_6H_5$,

or "benzoyl" group, is particularly important for anesthesia.

We now know that the power of producing local anesthesia is more common than any other physiological property among organic compounds, although the anesthetic property is an elusive will-o'-the-wisp, which easily may be lost when only slight changes in structure are made. Of the scores of new compounds created for this purpose during the period between 1890 and 1905, some had the desired action, and a few showed promise in surgery.

Eucaine, closely resembling the right-hand portion of the cocaine molecule, but of simpler structure, was widely employed for a time; it was followed by stovaine and alypin, but all of these rapidly fell into comparative disuse after discovery, in 1905, of novocaine, or procaine, as it is now officially called in this country. Procaine was first synthesized in Germany by Einhorn, and tested clinically by Braun.

Procaine.—The importance of the discovery of procaine can scarcely be overemphasized. It possesses many of the advantages which cocaine lacks; it is much less toxic than the latter, stable in solution, less expensive to manufacture, and does not lead to habit formation. When injected in conjunction with epinephrine, procaine produces local anesthesia practically equal to that of cocaine. Within a short time after its introduction, procaine was generally adopted as preferable to cocaine for injection, by the medical and dental professions throughout the civilized world. During the World War, it was designated by the Committee on Synthetic Drugs of the National Research Council as one of the three most valuable and indispensable drugs. During

the war and since, it has been manufactured in this country, at a low cost and of excellent quality.*

The borate of procaine base, under the name borocaine, has recently been suggested in place of the hydrochloride, which is the salt ordinarily employed; the basis of this lies in the fact that the borate is slightly alkaline, and solutions of salts of local anesthetics have long been known to increase in efficiency as we pass from an acid towards an alkaline reaction. The efficiency of borocaine in clinical practice apparently has not yet been definitely established.

Procaine fails almost completely in an important field in which cocaine is effective, namely, in the property of producing anesthesia by topical application—the so-called surface anesthesia. A crystal of procaine, placed upon the tongue, or a solution instilled into the eye, produces but little anesthesia. The use of cocaine for operations of the eye and nose, and in other areas, for a long time was not seriously challenged by any other local anesthetic which had been produced. During the decade following the discovery of procaine, many new local anesthetics were prepared but all of them

*The chemical formulas of some of the more common local anesthetics are given below, for purpose of comparisons:

Procaine $NH_2C_6H_4COOCH_2CH_2N(C_2H_5)_2.HCl$

Apothesine $C_6H_5CH=CHCOO(CH_2)_3N(C_2H_5)_2.HCl$

Butyn $NH_2C_6H_4COO(CH_2)_3N(C_4H_9)_2.\frac{1}{2}H_2SO_4$

Tutocaine $NH_2C_6H_4COOCH(CH_3)CH(CH_3)CH_2N(CH_3)_2.HCl$

failed in comparison with procaine and cocaine. The next important advance did not come until new alcohols were produced in quantity in this country, most of them as the result of the necessities of the war. These alcohols constituted the building stones from which other new local anesthetics could be formed. As a by-product in the manufacture of acetone, needed for explosive manufacture, came normal butyl alcohol; another alcohol, trimethylene glycol, was already available as a by-product of the soap industry. These and other intermediates furnished the starting materials for a renewed attack, in American laboratories, on the problem of ousting cocaine from its last stronghold.

Butyn.—The result of these efforts, extending over several years prior to 1920, was the discovery of butyn, a product related in structure to procaine, but somewhat more complex. Butyn, when instilled into an area such as the eye, is more effective than cocaine, and in addition, is free from certain drawbacks of cocaine, such as the property of causing drying of the cornea and dilation of the pupil. In addition, butyn is much more effective than procaine, when the solutions are injected, and anesthesia is produced more promptly—the latter quality being of particular importance in dentistry. Butyn, weight for weight, is more toxic than procaine, but because of its greater efficiency, it is employed in considerably lower concentrations. Like procaine, butyn is not habit-forming.

Other Synthetic Local Anesthetics.—Cocaine, eucaine, stovaine, procaine, butyn and most of the other local anesthetics that have come into extensive use, are derivatives of benzoic acid, but esters of other aromatic

acids may also exert marked anesthetic activity. During the early years of the war, when procaine was obtainable with difficulty, two American chemists took up the investigation of esters of cinnamic acid and from this research resulted apothesine. The anesthetic properties of this product resemble those of procaine quite closely. It is somewhat more efficient than procaine, and also more toxic; and like procaine, it is employed in infiltration anesthesia, rather than for topical application.

Two newer anesthetics have attracted attention abroad. One of them, called tutocaine, is also related to procaine, but is somewhat more complex. It is more toxic than procaine, but produces anesthesia in lower concentrations, and is more effective than procaine as a surface anesthetic. The other anesthetic, psicaine, is of interest because of its close relation to cocaine, the ancestor of local anesthetics. Cocaine, because of the intricate arrangement of atoms in its molecule, may exist in eight stereoisomeric forms; all of these eight forms have exactly the same atoms, linked to each other in the same order, but differently arranged in space. Natural cocaine is one of these, and psicaine is another. Psicaine is a synthetic product, discovered by Willstätter, who did so much to elucidate the constitution of cocaine itself. An interesting item with reference to this product is that it is stated to have anesthetic properties similar to those of cocaine but to be free from the tendency towards habit formation.

The Rôle of Epinephrine.—It is an interesting fact that chance or necessity frequently brings about the practically simultaneous discovery of two types of prod-

ucts, one or both of which would be sharply limited in usefulness without the other. So it has been with local anesthetics and epinephrine, or adrenaline. The American discovery and isolation of epinephrine from the suprarenal glands of animals at the turn of the last century by Abel, Aldrich, and Takamine, has had a profound bearing upon the applications of local anesthesia in surgery. The advantages of a local anesthetic lie partly in the localization of its physiological effects to the particular area to be treated; but the tendency is for the circulatory system to bring about rapid diffusion of the injected substance. This shortens the anesthesia and increases the danger of toxic effects, due to too prompt absorption of large quantities of the drug. These processes are prevented or delayed by epinephrine.

Epinephrine produces constriction of the blood vessels, and when administered with a local anesthetic, it delays absorption, decreases toxicity, increases the duration of anesthesia, and decreases hemorrhage. Cocaine itself causes some constriction of the blood vessels, but even with cocaine, it was frequently found advisable to employ epinephrine. As synthetic local anesthetics ordinarily exert little or no constricting action, the use of epinephrine in conjunction with them is often of particular importance. The abandonment of some local anesthetics, such as the eucaines, has been partly due to their incompatibility with epinephrine.

Other Types of Anesthetics.—Einhorn made the statement that esters of all aromatic acids exert anesthetic action, but this property is by no means limited to even this broad class of compounds. Phenacaine, or

holocaine, is a rather toxic anesthetic derived from acetphenetidine (phenacetine), and used to a limited extent in eye work. In addition, many inorganic salts, phenols, bases, halogen compounds and aromatic alcohols exert anesthetic action. Belonging to the latter class are benzyl alcohol, phenyl ethyl alcohol or rose oil, and saligenin, all of which have recently been proposed as of possible use as local anesthetics; but none of the members of these various classes has been found to compare favorably in all respects with the synthetic local anesthetics already described.

Insoluble Anesthetics—Dusting Powders.—There is, however, another class of local anesthetics which has found a distinct field of usefulness, not in surgery, but in general medicine. This comprises the water-insoluble local anesthetics, which are employed in various forms, such as dusting powders and ointments for the relief of pain due to denuded skin areas, in troches and sprays for nose and throat, and internally for the relief of pain due to gastric ulcer, etc. They are relatively simple chemical compounds, and of very low and practically negligible toxicity.

The first of these insoluble local anesthetics, anesthesin, or benzocaine, was discovered by Ritsert in 1890, even before the first of the injection anesthetics was synthesized. Anesthesin was originally not looked upon with favor by the German manufacturing firm which had taken over the product, and it was not until ten years later that the value of the product came to be recognized. To-day, thousands of pounds are employed annually in the United States alone. A similar preparation is orthoform, which was introduced in 1896, but

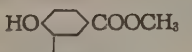
the use of this product is not free from occasional side reactions.*

It is well known that the different members of a series of compounds are likely to resemble each other in the kind of physiological action which they may exert, but not in the degree of such action. So it is with anesthesin and its homologs. As we go up the series from the ethyl ester, which is anesthesin, through the higher esters, propyl, butyl, isobutyl, amyl, etc., the efficiency first rises, then rather sharply falls off again. The maximum efficiency of the series is found in the normal butyl derivative called butesin, which is over four times as effective as anesthesin. The availability of butesin has led to the introduction of a new type of product, in which both antiseptic and anesthetic properties reside to a marked degree in a single compound: butesin picrate, because of this combination of therapeutic properties, is useful in the treatment of burns and other denuded skin areas.

Future Local Anesthetics.—Synthetic organic chemistry has thus succeeded not only in preparing excellent substitutes for cocaine, which are less toxic, less expensive and not at all habit-forming; but it has also given medicine special local anesthetics for specific purposes—such as procaine and similar products for deeper

*The formulas of some of the more common water-insoluble local anesthetics are given herewith:

Anesthesin (Benzocaine)

Orthoform New 

Butesin

surgical operations, butyn and related compounds for use on mucous membranes, and finally anesthetics for the preparation of ointments to be used on burns, ulcers, etc. As the important advantages of local anesthesia have impressed themselves, during the last two decades, upon physician and dentist and patient alike, the applications have increased by leaps and bounds, until today there are few operations which cannot be performed in this manner by the skilled operator. In dentistry, general anesthesia has fallen far behind the local method; and in many operations in general surgery, as for example in tonsillectomy, local anesthesia is attracting an ever increasing number of followers. To the eye, ear, nose and throat specialists, the advent of cocaine and its successors has been of inestimable advantage.

The ideal local anesthetic, among other qualities, should be of low toxicity in the concentration employed; it must be stable in aqueous solution, and sterilizable by heat; non-irritating and painless; compatible with epinephrine; and it must be efficient in dilute solutions. While this ideal has not been fully attained in any local anesthetic, some of the products at present available closely approach the ideal in their practical aspects. From the very nature of anesthesia, all toxic characteristics and all danger cannot be eliminated; but the millions of cases in which local anesthesia is annually employed attest to its merits.

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CHAPTER VIII—ARTICLE 4

FIRST AID FOR INSOMNIA

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Assistant Professor of Chemistry,
University of Chicago

Dr. Rising, as Assistant Professor of Chemistry at the University of Chicago, has made a special study of synthetic drugs in connection with her research in organic chemistry. During the war-shortage of synthetic drugs, she helped to develop an important method for preparing luminal, a hypnotic widely used to prevent the seizures of epilepsy, for which there was a most pathetic need in 1917-18. The method was immediately published for the benefit of any manufacturer. Dr. Rising is making a study at the present time of the physiological effect of optically active hypnotics as compared with that of the optically inactive form of the drug.

In days well within the memory of living physicians, nearly all of the most useful drugs were obtained from natural sources, morphine from poppy seeds; quinine, used as a specific in the treatment of malaria, from the bark of the cinchona tree of Peru; digitalis, a heart stimulant, from fox-glove; it is unnecessary to multiply examples. Some of the drugs provided by Nature still hold first place in usefulness, while others have been replaced by products created in the chemist's laboratory, the synthetic drugs of modern times. In many instances there have been invented medicinals which possess the desirable physiological behavior of the naturally occurring substances but lack their toxic properties. Thus cocaine, obtained from the leaves of the coca plant, has been largely replaced as a local anesthetic by the synthetic procaine, which is far less toxic than is cocaine. Again, the chemist has been able to synthesize remedies,

such as the arsenicals, of which there are no naturally occurring prototypes. Synthetic drugs have become valuable implements in the hands of the physician, and so it is that chemist and doctor have cooperated in the humanitarian spirit of an age which is so manifestly characterized by the urge to turn to practical account the discoveries of science in order to promote the happiness, health, and well-being of all the peoples of the earth.

Insomnia.—Among the synthetic medicinals which have been created to meet specific needs are the hypnotics, or sleep-producing substances. The ailment insomnia is a common one; it is frequently the result of pathological conditions such as nervous diseases, deep-seated digestive disturbances, or other organic disorders of a serious nature. All of these troubles are in turn augmented by the continued loss of that vital necessity for health, sleep. The dangerous nature of this "vicious circle" is fully appreciated by the physician, who frankly dreads its occurrence and results, and recognizes that persons afflicted with a marked and persistent insomnia are sick and await a cure. In the old days the sufferer from sleeplessness or other disorder of a nature requiring for relief the administration of a sedative, was quieted and brought into a health-giving sleep by the use of a preparation of opium, or one of its active principles, morphine or codeine. Later, chloral became available, following the observation in 1869 of its sleep-producing property. Chloral had been discovered as early as 1832, but for nearly forty years its humane sedative behavior was unknown. In cases without number the condition of relaxation which precedes the curious phenomenon of sleep has been induced by these

drugs. But always in prescribing morphine and chloral, the physician runs the grave risk of habit-formation in the patient, even when small and infrequent doses are used. These and other untoward properties of the older sedatives have served as a stimulus to the synthetic chemist to devise and prepare for the physician the modern hypnotics, the goal being to attain the maximum sedative action with a minimum of dangerous secondary effects. As a result the physician has today a long list of safe hypnotics from which to choose the one best adapted to the needs of his patient. These drugs are specifics for sleeplessness, just as diphtheria antitoxin is a specific for diphtheria, and quinine for malaria. They are excellent sedatives which have been used successfully and with relative safety to relax tense nerves and cause sound sleep of a far more refreshing nature than that induced by any opiate. When wisely and rightly used, the modern sedatives are not unduly toxic nor dangerously habit-forming. Without question the discovery of hypnotics and their adaptation to human wants has proved to be one more aid to health in a needy world.

Hypnotics.—A brief consideration of the meaning of several terms which are necessarily embodied in a discussion of hypnotics should enable the reader to feel at home if perchance the present subject is foreign to his interest and experience. Among sedative drugs there are *narcotics, anesthetics,* and *hypnotics.* The sedative effect is common to the members of all three groups. What, then, are the differences in behavior upon which this classification is based? The term narcotic, as commonly used, refers to the opiates, such as morphine, heroine, and codeine.* These differ from many sedatives in that

*Cocaine is also classified as a narcotic in the Harrison Federal Narcotic Act.

they produce a condition of analgesia, that is, they render the body more or less insensible to pain. Morphine and heroine are dangerously habit-forming, codeine somewhat less so, and this behavior is highly characteristic of narcotics. Anesthetics, like narcotics, produce analgesia, but of relatively short duration—a matter of minutes with nitrous oxide (laughing gas), and of a few hours at most with ether or ethylene. To administer these agents in a sufficient amount to produce a more lasting anesthesia would be most unsafe, whereas the opiates may be used in such a way as to confer insensibility to pain over a period of many hours. Hypnotics resemble narcotics and anesthetics in that they produce sleep, but a typical hypnotic does not stop pain, and herein lies its outstanding difference from other sedatives. When there is the combined need of sleep with relief from pain a narcotic is usually prescribed, or a combination of a hypnotic with a non-narcotic analgesic, of which a few of rather limited application have been produced synthetically. Since there are many insomnias which take their origin from nervous disorders unaccompanied by pain, the non-analgesic hypnotic has an important place among modern drugs and has been a boon to many a tormented patient.

To one interested in the development and growing importance of the highly specialized branch of chemistry which is concerned with the production of synthetic drugs, the history of the creation of hypnotics forms a representative chapter of an inspiring tale of scientific achievement. Let us scan the succession of events which has led to the discovery of substances hav-

ing the valuable physiological property of producing hypnosis.

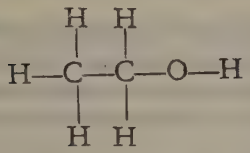
Sleep Produced by Chemical Influences.—The actual production of hypnotics in the laboratory has been preceded by careful consideration on the part of the chemist of certain fundamental problems related to the matter in hand. One of the most important of these has to do with what may be called the chemical "why" of hypnosis. The chemist begins his work with an intensive study of substances of known sedative effect, in the effort to discover the chemical cause of this behavior—a matter of profound importance. When he finally recognizes and understands the factors which are involved in the sedative action of typical known hypnotics, he will be able to construct his own medicinals with a maximum of intelligence, embodying in them attributes known to be conducive to hypnotic effect, and enhancing this property by modifications of his own devising.

Undoubtedly some of the contributing causes of hypnosis are as yet undiscovered, but certain valuable information has been obtained which every inventor of new sedatives utilizes. The first of these underlying factors in the production of sleep is inherent in what may be called the chemical nature of hypnotics. In the term chemical nature there is implied the kinds of matter (elements) which are to be found in the molecule of a hypnotic substance, and the way in which the atoms of these elements are arranged in a single molecule. Chemical nature is distinct from physical nature, which has to do with more superficial aspects of the behavior of substances, such as their solubility, state (gas, liquid, or solid), and tendency to vaporize or solidify. Both

the chemical and physical nature of sedatives contribute to their specific effect upon the body, as will be seen.

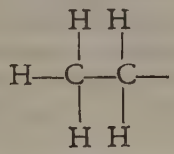
Bromides.—It is a striking fact that a great variety of substances behave as hypnotics. Among the simplest, chemically speaking, of all the known sedatives are the bromides, sodium bromide and potassium bromide. The chemist designates these with the utmost brevity as NaBr and KBr, respectively, using the symbols Na for sodium (natrium in Latin), K for potassium (kalium), and Br for bromine. These bromide salts are chemical cousins of the sodium chloride (NaCl) with which we "salt" our food. Of the three salts mentioned, only the ones containing the element bromine are sedatives, and the sedative effect is ascribed to the bromine, this conclusion being amply supported by the sedative behavior of many other substances containing the element bromine. The bromides are standard among the milder sedatives. They are preeminently useful for nervous disorders for which a powerful hypnotic is not needed, as in neurasthenia and hysteria. They are also used in the treatment of epilepsy, and, in large doses, for convulsions. Fortunately, bromides are not dangerously toxic, nor habit-forming when administered in small doses, even over an extended period of time.

Alcohol.—One of the very oldest members of the hypnotic family is ethyl (grain) alcohol. Alcohol was presumably the only hypnotic known in earlier centuries besides opium. The ultimately stupefying effect of the wines, meads and ales of those days was caused by the ingredient ethyl alcohol. The chemist designates ethyl alcohol by the formula—



which tells him that its molecule is made up of atoms of the element carbon (C), hydrogen (H), and oxygen (O). Chemically speaking, ethyl alcohol is wholly unlike the bromides; for one thing, the molecules of alcohol and the bromides do not contain the same elements. Yet physiologically they produce the same effect, that of hypnosis. Can the discerning chemist associate with any atom or group of atoms in the molecule of ethyl alcohol the hypnotic properties of the substance as he was able to do in the case of the bromides?

It has long been a matter of observation that many substances whose molecules contain the group of atoms



or C_2H_5—, called by the chemist an ethyl group, show a marked affinity for the nerve tissues of the body. One instance of this behavior is found in the hypnotic effect of many substances which, like ethyl alcohol, possess one or more ethyl groups. Other hypnotics of this type will be discussed presently. The selective action upon the nervous system of molecules with ethyl groups is also shown in the behavior of certain dyes with molecules containing ethyl groups. These dye nerve fibers, while the same dyes with methyl (CH_3—) groups replacing the ethyl fragments have no such effect.

Ethyl alcohol itself is seldom prescribed as a hypnotic nowadays. Its deleterious effects upon the body, toxicity, and the danger of habit-formation attendant upon its frequent use are too well known to need emphasis here.

Urea.—Another substance which possesses sedative properties is urea. Urea is an important by-product of life processes, and is produced in our bodies day by day as waste material from the utilization of protein food by the organism. This waste material is extracted from the blood by the kidneys and excreted in rather large amounts in the urine. Urea is chemically unlike the bromides and ethyl alcohol. Its formula,

$$H_2N.CO.NH_2$$

tells us that the molecule contains the element nitrogen (N), besides carbon, hydrogen and oxygen. Urea itself is such a mild sedative that it is not useful as a hypnotic, but certain of its derivatives constitute some of the most powerful and useful hypnotics known. We shall presently turn our attention to these important derivatives of urea.

A Basis for Progress.—This examination of the chemical nature of three representative members of the hypnotic family has failed to reveal to the chemist a source of their hypnotic effect which is common to all three of the substances. But observations of the type just discussed led him to investigate the physiological behavior of some related compounds. He found to his delight that other bromides besides those mentioned are sedatives; indeed, the behavior of the alkali bromides is typical for bromine derivatives in general. Moreover, he took careful note of the fact that many substances

containing in their molecules the arrangement of atoms known as the ethyl group, as found in ethyl alcohol, are sedatives. The similar behavior of urea derivatives has already been emphasized. One is forced to the conclusion that the bromine atom, the ethyl group, and the urea molecule are specifics for the production of sleep. These discoveries have been remarkably stimulating to the creative chemist. With the definite intention of constructing hypnotics he has synthesized molecules containing one, or more than one, of these powerful groups, and today there exists a great number of substances of marked hypnotic power, the production of which is the direct result of the recognition of these sources of sedative action. In such discoveries is embodied the best answer which has so far been found, from the angle of chemical nature, as to the "why" of hypnosis.

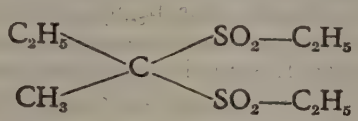
Physical Factors.—In comparatively recent times evidence has accumulated which emphasizes the influence of the physical nature of hypnotics in determining sedative action. Among the requisites of sedatives which pertain to their physical nature is solubility; it is by no means the only physical property which must be taken into account, but it is among the most important. Obviously enough, a substance which cannot dissolve readily in the body fluids is of little value as a drug, hypnotic or otherwise. Since sedatives act within the nerve cells, they must penetrate (dissolve in) these to be effective. The nerve tissues are made up in part of fats and fat-like materials and in part of water, therefore a hypnotic must be soluble both in fat and in water. The results of research thus far have shown that the efficiency of a hypnotic is directly proportional to the ratio of its solubility in fat to that in water.

The influences of chemical and physical nature upon hypnotic behavior have been discussed separately, but it should be pointed out that they are interactive. The chemical nature of a substance largely determines its physical nature, for instance, its solubility. The presence of an ethyl group in a given molecule may increase the solubility of the substance in the nerves, thus contributing to the hypnotic effect by influencing its physical nature.

Creative Chemistry.—For a chemist the occupation of producing molecules, of fitting atoms and groups of atoms into a harmonious whole, is a rare sport. He is an artist, whose colors are the atoms; and while his completed picture may appear to the uninitiated so extremely unintelligible that the layman consigns it coldly to the impressionistic or even futurist category of art, there is innate warmth of concept and beauty of line for him who truly understands. It is to acquaint the reader with a little of the technic of the chemist-artist that a few of his masterpieces in the field of hypnotics will now be described.

Chloral.—Chloral was the first synthetic hypnotic, and was exceedingly important for its time since it often replaced morphine when simply hypnosis, without analgesia, was sought. But chloral is injurious to the body in a number of ways: it is distinctly habit-forming; it is harmful to the heart, causing cardiac depression; it is irritating to the mucous membrane which lines the stomach and intestines, and the danger attendant upon its long-continued use is obvious for this reason alone. Chloral has not maintained its early high rank as a sedative, and the physician of today prefers, in the majority of cases, to use the safer modern hypnotics.

The Sulfonals.—The "sulfonals," sulfonal and trional, were the first important synthetic substitutes for chloral. These are organic (carbon) derivatives of the element sulfur. Trional has the structure



The repetition of the hypnotic ethyl group is noteworthy; the use of this expedient has enabled the chemist to fashion a molecule having a pronounced sedative action. Moreover, the sulfonals are cheap to manufacture, and this is an important item in the production of any drug on a commercial scale. Some physicians prefer these effective hypnotics to all others, but it is a recognized fact that the use of the sulfonals is not unaccompanied by dangers. The drugs are not decomposed to any extent in the body, and are slowly excreted unchanged in the urine. This elimination occurs so gradually that the drugs tend to accumulate in the system when used steadily; presently the organism becomes acutely poisoned and under these conditions death is said to follow in a large majority of cases. In occasional doses the sulfonals are safe, but if used on two or three consecutive days the administration should be interrupted to allow complete elimination, some other hypnotic being substituted meanwhile, if necessary. Usually the sulfonals bring on a restful sleep promptly, perhaps within the hour; but occasionally it is delayed till the next day, an obviously inconvenient occurrence! Use of these sedatives leads sometimes to habit-formation. It

is evident that the sulfonals are not wholly satisfactory as hypnotics.

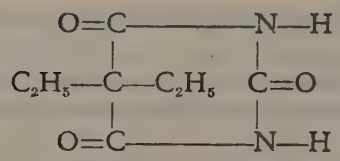
An important step in advance in the production of safe hypnotics was taken when an intensive study of the derivatives of urea was begun. It will be recalled that urea has itself too mild a sedative action to be of therapeutic value. If sleep is partly the result, as some believe, of a mild poisoning of the body by its waste products, then the day's accumulation of urea may play its part as a natural hypnotic in producing the night's much needed rest. As urea is native to the body and therefore readily tolerated by it, it was reasonably surmised by the chemist that the sedative powers of urea might be enhanced by the introduction into its molecule of other sedative groups without an undue and dangerous increase in its toxicity.

Toxicity.—This matter of the toxicity of his products is one of the most vital concerns of the drug chemist. A synthetic substance may possess most excellent and gratifying sedative properties and be at the same time a rank poison! The ideal hypnotic must show a convincingly sedative behavior and be at the same time so little toxic that the body can tolerate it not only in occasional doses but for prolonged use when imperative need arises. Practically all substances foreign to the body, that is, materials not normal ingredients of the blood or tissues, nor yet foods, are toxic to a greater or less degree; exceptions to this rule are rare indeed. Hence it cannot be predicted with any degree of certainty whatever that a given synthetic product will be without toxic effect. The most that can be hoped for is a minimum degree of toxicity. The margin of safety between what may be called the curative dose of a drug

(the sleep-producing dose in the case of a hypnotic) and the dangerously toxic dose should be as large as possible. Dr. Paul Ehrlich, of salvarsan fame, that giant among the master minds that have contributed to the development of synthetic drug chemistry, aimed at a curative to toxic ratio of one to three for his arsenicals. For some of the important hypnotics an even greater margin of safety has been achieved.

Ureides.—The hypnotic derivatives of urea, classed as ureides, are modified ureas in which an intensification of the sedative behavior of urea has been accomplished through the introduction into its molecule of atoms or groups of atoms of known sedative effect. One interesting ureide, called by the chemist diethylbromacetylurea, combines in its molecule, as the name implies, the three important hypnotic groups, ethyl, bromine and urea. Its formula is $(C_2H_5)_2BrCO(NH.CO.NH_2)$. This substance, first produced under the name of adalin, is listed in the United States Pharmocopeia as carbromal. It is useful in the treatment of the milder nervous diseases, being somewhat less powerful in its physiological effect than are the other ureides, in spite of the multiplication of sedative groups in its molecule. Carbromal appears to be reasonably free from undesirable toxic effects and has been successfully used in cases where a powerful hypnotic is not required.

Among the hypnotic ureides the one introduced as veronal, and designated in our Pharmocopeia as barbital, is probably the most valuable. Veronal was first prepared in 1903 in Germany by Emil Fischer, one of the greatest chemists of all time. Inspection of the formula of barbital reveals the presence of a urea group and two ethyl fragments:



Barbital ranks very high as a safe and efficient hypnotic, and is more generally used than is any other non-analgesic sedative. It is powerful, can be depended upon to act promptly to produce a deep, restful sleep, and seems not unduly toxic when moderate quantities are used; the dangers associated with a frequent and prolonged use of barbital and other hypnotics will be discussed later. Excessive doses of barbital cause death. With some few persons it has a marked depressant action, even in small amounts, and in such occasional instances its use is contraindicated. Barbital is also on the market in the form of its sodium salt as "barbital-sodium," and "medinal." The salt has the same physiological effect as has barbital itself, but some physicians prefer it to barbital since it acts more promptly on account of its greater solubility.

Another ureide, closely related in chemical nature to barbital, was introduced as luminal, and is known in America as phenobarbital. The drug has proved to be a satisfactory agent in the treatment of numerous nervous troubles. On account of its specific action upon the section of the brain known as the motor area, which controls nerve impulses to the muscles, phenobarbital is peculiarly suitable for the treatment of diseases of the nervous system in which it is desirable to control muscular spasm, as in petit mal, commonly known as epilepsy. The drug serves both to prevent and to alleviate

epileptic seizures. It is useful also in the treatment of St. Vitus' dance.

The intrinsic worth of phenobarbital was shown with remarkable vividness during the World War. Before the war luminal and other important synthetic medicinals were produced only in German laboratories, whence they were exported to this country. When America was cut off from that source of supply by the exigencies of the war, a drug famine promptly overtook the country. Expert chemists working in the research laboratories of drug manufacturers and of universities at once undertook to devise practical methods of synthesis for hypnotics, arsenicals, local anesthetics and other wanted drugs.

A study of the preparation of luminal was undertaken in Kent Chemical Laboratory at the University of Chicago. As the news of this work travelled over the country, many pitiful calls began to come in from doctors, epileptics, and, most pathetic of all, from parents of epileptic children, begging for small amounts, any quantity, of the sedative. Sadly enough, it was not possible to supply these urgent needs, since the drug was prepared on a very small scale in Kent Laboratory. Only months after the close of the war were American manufacturers able to provide sufficient quantities of the drug to meet all needs.

This bit of synthetic drug history is related here in order to emphasize several important phases of drug research. It illustrates forcefully the humanitarian aspect of all work upon synthetic remedies. Moreover, it proclaims in no uncertain terms the imperative need that a great country like our own should be self-sufficient in the production of its medicinals, just as with foodstuffs

and any other vital necessities. The production of phenobarbital illustrates, too, some of the difficulties attendant upon war-time syntheses. The methods of preparation outlined in the patents were relied upon in the initial efforts to produce the synthetics; usually these procedures yielded enough of the desired product to be identified, and no more. This was a grave drawback to the adaptation of the patent methods for commercial purposes.

Combination of Hypnotic and Analgesic.—It was stated early in the discussion that hypnotics function as sleep-producing substances but are without the power to stop acute pain. In view of these facts, an important limitation to the use of hypnotics is readily discernible: they are of little value as aids for sleeplessness which is caused by severe pain. In early days the physician was often compelled to administer an opiate when it became necessary to relieve pain in order to produce sleep; nowadays in similar situations he very commonly finds it expedient to prescribe both a hypnotic and a synthetic analgesic to produce the double effect of morphine, and this procedure often brings most gratifying results. The drug sold as allonal is a readily obtainable combination of this sort, in which a hypnotic ureide, similar to barbital, is combined with pyramidon, an effective synthetic analgesic. Allonal, and other remedies of its kind, are far less dangerous from the point of view of habit-formation than is morphine, and have replaced this narcotic in instances without number. It is especially useful in post-operative treatment, for severe neuritis, and similar passing ailments. They are less useful in chronic conditions where there is constant and severe pain, as in cancer.

The sedatives described here are not the only efficient drugs of their kind, but are typical members of the hypnotic family, and among the most valuable. In the course of their production literally dozens of substances have been synthesized and tested for hypnotic properties. Relatively few of these have proved sufficiently valuable to warrant their introduction into medical practice. It may be of interest to record the fate of the vast majority of the literally dozens of potential hypnotics. Of the many prepared, only a few pass muster; the rest may prove when tested to be without hypnotic effect, or too toxic, or too costly to prepare. This is, indeed, the history of the production of all drugs by the synthetic method, which is essentially a method of trial and error. Such was the experience of Ehrlich, who sought long and patiently to produce a cure for syphilis, synthesizing six hundred and five arsenic-containing substances which proved to be of little or no value therapeutically, before he discovered the magic "606." These are the customary experiences of all synthetic chemists. Neither good hypnotics nor other valuable drugs spring full-fledged from chemists' test tubes.

Action of Hypnotics on the Body.—While the vital concern of the drug chemist is the actual production of drugs, he finds it important to study also problems related to their use and behavior. He has undertaken to discover, for example, how and where hypnotics act in the body, and their fate after the fulfillment of their destiny. Sedatives are taken by mouth and absorbed from the alimentary tract by the blood, which carries the larger part to the nerves. Conditions within the nerve cells are modified in such a way that excitability is reduced; little is known of the mechanism by which

this result is accomplished. There are in addition certain secondary reactions of sedatives upon the organs of the body, directed from the nerves. Respiration and heart action are extensively retarded, with the result that the normal supply of blood to the tissues is reduced. The body is thus forced to exist temporarily upon low rations of food and oxygen; moreover, waste products are not removed at the normal rate. These conditions are conducive to hypnosis, but have an undesirable effect upon the organism.

The sedative is not changed appreciably in chemical nature in the nerves. It is removed gradually by the blood, from which the kidneys extract it as waste material, to be excreted in the urine. Excretion is often nearly, but not quite, complete within twenty-four hours. A small part of the hypnotic remains longer in the body, and so it is that a single dose often serves for two nights. When the drug must be used for a longer time, its administration should be frequently discontinued for a night or two to prevent undue accumulation. Bromides and sulfonals are excreted more slowly than are the ureides; their accumulation is therefore more rapid.

Warning.—The beneficent aspect of the discovery of synthetic hypnotics has been emphasized, and rightly so; chemist, doctor and patient alike have reason to rejoice in the accomplishments of the past twenty-five years. The present discussion cannot be concluded, however, without an insistent warning with regard to the ill-advised use of sedatives. Such drugs, like any others, poison the body and should be used only in time of real need, and then only under the observation of a competent doctor. Hypnotics are habit-forming; they are

not so dangerous in this respect as are the opiates, but under no conditions should they be used in excessive quantities for an extended time. When the physician finds their continued use necessary, he ordinarily varies the kind so that addiction is avoided. It has been proved by experiment that the nerve tissues of the body may be irreparably injured by prolonged use of the ureide hypnotics. For these reasons many doctors, druggists and others in close touch with the use and sale of drugs deplore the fact that allonal, veronal, and the other ureides may be bought without a prescription, and believe that ultimately their sale will be restricted, as is the case with the opiates and cocaine. There is assuredly a legitimate use for hypnotics, but the present enormous sales indicate that many persons who are perhaps wholly unaware of the harmful physiological effects of these drugs when used in excess are doing themselves serious injury.

The story of hypnotic drugs related here is, after all, an unfinished one. Undoubtedly the splendid achievements of the past in that somewhat obscure but busy corner of the world, the chemist's laboratory, are but prophetic of future progress, and the drug chemist will continue to render there his service in the alleviation of the many ills to which human flesh is so pitifully heir. The production of sedatives will go on; perhaps some entirely new type will be discovered to replace even the ureides, the best hypnotics so far available. Fundamental problems related to hypnosis will be studied in the effort to determine the underlying cause of toxicity, and the results of such study will assure the production of remedies in which poisonous effect upon the organism is reduced to the vanishing point. It is

both cheering and stimulating to consider that work will be continued until a sedative agent having all the desired and a minimum of undesirable physiological properties is at hand—till the ideal of the hypnotic chemist has become a reality.

Chapter VIII—Article 5

CHEMISTRY TO THE AID OF THE HEART

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Professor Hatcher, after a brief connection with the school of medicine of Western Reserve University (1900-04) has been on the staff of the Department of Pharmacology of Cornell University Medical College since 1904 and professor of pharmacology since 1908. His noteworthy contributions to the study of quinine and strychnine and more particularly of digitalis have given him an authoritative voice on remedies for heart disease. For almost a quarter of a century he has been an active and influential member of the Council of Pharmacy and Chemistry of the American Medical Association.

Many of those for whom this book is intended are not versed in physiology or pharmacology, hence a few statements of an elementary nature will help to a better understanding of the discussion.

The heart is often called a pump, and few who have not become especially interested in one of the problems concerned with the circulation understand the complexity of the many questions involved in the physiology, pathology and therapeutics of this important organ. Every one knows that the heart has four chambers; of these the right auricle receives blood from the veins and passes it to the right ventricle, which pumps it through the lungs. From these the blood returns to the left auricle and is transmitted to the left ventricle, which pumps it to all the organs and tissues of the body. Stated in this way the work of the heart seems extremely simple, but the many processes involved in the work of the heart

have long furnished many of the most difficult problems in physiology, pharmacology and therapeutics.

When one reflects that the beat of the heart cannot suffer interruption for a single minute from the moment of birth before death occurs, one appreciates the importance of the maintenance of the heart in a state of health. The brain has periods of relative rest during sleep, and even during waking at times, but while the work of the heart varies to an extraordinary degree with changes in mental and physical activity, even during sleep it is imperative that it shall maintain an adequate circulation. The very fact that the work of the heart must change with every change in mental and physical activity makes the mechanism of its control all the more complex. It is almost as necessary that the heart shall beat with less energy when a lessened volume of circulating blood per minute is required as it is that it shall beat with greater energy when increased activity demands that a greater volume be sent to the tissues. A sudden severe effort is impossible when the heart is incapable of a sudden and corresponding increase in the energy of its beat, and in order that these varying demands shall be met promptly the heart through the nervous system is in relation with every organ and tissue of the body, and it is also in relation with many organs by means of substances carried by the blood.

The circulatory system is extraordinarily complex, and anything that injures any organ affects the heart eventually. When the arteries become less elastic, as they do with advancing years, they are incapable of carrying as large a volume of blood as previously without increased pressure, hence the heart must then work with greater vigor. That would exhaust the average heart in

a short time, and we find that the elderly are compelled to limit their exertions. Unfortunately, the demands of life often compel the old to work beyond their normal capacities, and the inclinations of many cause them to pursue lives that have the same effect on the heart, which is unable to stand the strain imposed and it fails gradually, or rapidly. Despite the obvious importance of the healthy condition of the heart there was no scientific treatment of cardiac disease until within a few years past. Much of the treatment formerly did more harm than good, but justice requires that acknowledgment be made for the rare good sense of a few physicians such as William Withering, who treated patients with cardiac disease with great skill, considering the lack of modern methods and the advantages afforded by recent discoveries.

Drugs.—A brief explanation of what constitutes a useful drug will aid in the understanding of the present discussion. A drug is a substance that increases or decreases the function of one or more organs or tissues. It may do either of these primarily and then the other, but it is far more common for stimulation in some degree to occur first and to be followed by depression. The stimulant change varies from that which is hardly perceptible to violent convulsions, and from depression that is equally difficult to perceive to that causing paralysis and death. There are degrees of action between these extremes occurring simultaneously in different organs, or consecutively in one. Hence, while a drug produces only stimulation or depression, or both, there is limitless variety in the effects of the same drug with different doses, in different individuals and under different conditions in the same individual.

The therapeutic action of a drug is that stimulation or depression of a given organ or structure that is demanded by the condition of a patient in a given disease, or, more accurately, at a given stage of a disease, for the condition may change many times a day. The reader should be careful to understand just what is meant by therapeutic action as just defined, because every drug that enters the circulation is carried to every part of the body and actually produces some slight effect, at least, on every organ. These actions which are not desired are called side actions in contrast to the therapeutic effects, and since only one action is usually desired, it follows that there are commonly many side actions for one therapeutic effect.

In every locality there are plants that have therapeutic actions and primitive peoples use those that grow near at hand, but highly civilized people have the whole world from which to select their drugs and in addition they have the resources that chemical research has made available. There are hundreds of thousands of substances that exert therapeutic actions, but there are only about one hundred that are by common consent deemed essential for treating the sick, partly because by far the greater number have so many side actions that they are useless in therapeutics, partly because many are surpassed in their therapeutic actions by those in common use.

Every drug that enters the circulation must pass through the heart which is subjected to its action to some extent. This is negligible in most cases, but there is a tendency even among many physicians to lose sight of the fact that the heart is injured seriously by many drugs that are administered for their therapeutic action on other organs.

Only a few of the essential drugs are necessary in the treatment of cardiac disease even though the heart is capable of being influenced in many ways. We seek always the ideal drug, that is one that comes nearest to inducing just the therapeutic action desired with the least of the side actions. There is no ideal drug, for the reasons stated, and at times we must sacrifice much to the urgency of the case and use a substance that promptly gives the desired effect even though it causes undesired actions temporarily. There is a limitless field for the exercise of chemical research for the purpose of finding substances that will cause a maximum of the desired effects on the heart, with a minimum of the side actions.

When the heart beats feebly a stimulant is administered to increase the force of its contraction or the frequency of the beat, or a depressant is used to lessen the work that the heart is compelled to perform. If it were not for the harmful side actions of various drugs it would be a comparatively simple matter to continue the administration of any one of several that have the necessary therapeutic action until the desired effect is induced. The problem is not nearly so simple, however, and it is never possible to know just how much will be required in any case. If one does stimulate to the desired degree one must be extremely careful to avoid overdosage that will later make matters worse, for the effects induced are often delayed for some days during which the administration of the drug must be continued.

Pure Principles; Quinine and Quinidine.—Even the purest principles, such as quinine and quinidine, both of which are obtained from cinchona, or Peruvian bark, act injuriously on many organs unless the dose is regulated with precision. Peruvian bark contains some

twenty alkaloids or more, and in addition a high percentage of tannin which is irritating to the stomach and which interferes with the absorption of the quinine in the bark or the tincture made from it. Peruvian bark was used for the cure of malaria during nearly two centuries before quinine came into common use, and one may ask why we do not use it today. It was a wonderful discovery for those who had no better remedy against malaria that claimed millions of victims, but we hear little today of the many failures that attended its use. No two specimens of Peruvian bark contain the same amounts of quinine, and furthermore, the rate of absorption varies with many different conditions. Hence it was impossible to know just how much any patient needed; consequently he was usually given about as much as it was thought that he could stand without injury. Sometimes the amount of quinine absorbed was insufficient and the patient was not cured; sometimes he absorbed more than he required and he suffered injury to the organs of hearing. Such a hit-or-miss method of using quinine or quinidine for the relief of auricular fibrillation ("quivering" of the auricles) is out of the question, for an overdose may cause death. Every patient who suffers with auricular fibrillation and gains relief through the use of quinine or, still better, quinidine, is indebted to chemistry for the isolation of these alkaloids.

We have been accustomed so long to using pure quinine and quinidine in a high state of purity that we are inclined to lose sight of the debt that we owe to chemistry for their preparation. The chemist was compelled to devise means of separating the quinine and the quinidine from the many other substances which

are extracted together from the bark. This was no simple task because he did not know what he was looking for; hence he could not know what the physical and chemical properties of the substance sought were. The problem was not at an end when quinine and quinidine were finally isolated, for different specimens of the bark contain different constituents, and it is probable that no two specimens of quinine or quinidine are prepared in exactly the same way, though the ultimate product is exactly the same whatever the method of preparation employed by the chemist.

Though the chemist started the investigation of Peruvian bark without knowing the nature of the substance that he sought, he soon found alkaloids which are stable and from which salts are formed with acids. From these salts the bases may be precipitated with alkalies, and since the different salts and bases have different solubilities in various solvents, it was relatively easy to prepare the pure alkaloids quinine and quinidine. It is only relatively easy, however, for even this requires great skill, and one of the finest exhibits ever made by a certain prominent firm of manufacturing chemists at a meeting of the American Medical Association consisted of large crystals of pure alkaloids and their salts. Comparatively few even of the physicians who saw the exhibit appreciated its importance and the skill that made it possible. The present writer has always held that firm in greater esteem because of that exhibit.

The contribution of chemistry to the relief of the heart by quinine and especially quinidine has been considered at some length because it is a comparatively simple problem, and the main facts are presented so that the layman can appreciate the debt that we owe to chem-

istry in this field. The discussion of the most important group of heart remedies involves so many technical questions that even those who have devoted years of study to the subject cannot pretend to understand them all. It is necessary, therefore, to omit many details in this extremely difficult field, but such is the importance of the problems that the difficulties merely serve as a spur to further efforts. Everyone who finds himself baffled for the moment believes that ultimate success will be achieved, and it is certain that success will justify all the labor that will have been expended in the search.

Digitalis.—The principal drugs that are used for the relief of the failing heart are commonly called digitalis bodies, because digitalis is the best known among the group and the essential therapeutic action is the same in all. Unfortunately, every one of them has many side actions, no two have the same total constituents or any single constituent in the same proportions: even different specimens of digitalis leaf have different amounts of active constituents. Digitalis (foxglove) gathered at one season of the year differs from that gathered at another; that grown in one locality differs from that grown in another; the leaf of plants grown in the same soil and gathered at the same season may show differences from year to year. In short, man has not discovered any means of producing digitalis of uniform activity or uniform proportion of active constituents.

One may ask why digitalis and other related drugs of even less usefulness can be used therapeutically if they present difficulties analogous to those of Peruvian bark, but even greater, in view of the fact that Peruvian bark cannot be used instead of quinine or quinidine in

auricular fibrillation, though, as previously stated, it was long in use in a hit-or-miss fashion in the treatment of malaria. Digitalis was used for more than a century in the treatment of cardiac disease with no greater precision than that with which Peruvian bark was used in malaria, and many deaths resulted because of a want of knowledge of the active constituents and the proper dosage. Some died from heart failure because they did not receive enough digitalis to induce the desired effects, others died from overdosage. Chemistry had been unable to isolate the essential pure principles, and some of those principles that were isolated early in the study were unsuited for oral administration, and the proper technic of using them by injection into a muscle was not known.

Desperate conditions often justify desperate remedies, and when a physician has a patient who will surely die within a few hours if relief is not afforded he may be compelled to adopt a measure that sometimes leads to recovery and sometimes results fatally. There is probably no physician who has been in general practice during many years who has not found himself confronted by such a situation more than once. Formerly the physician whose patient suffered with cardiac disease of such serious nature had no way of knowing the activity of the digitalis that he was compelled to use. He was forced, therefore, to administer a dose that in his judgment was best—sometimes it was just right, but often it was too little or too much, and in either of these cases the result was a tragedy. Stress is placed on this uncertainty concerning the activity of digitalis, but despite all its disadvantages it was, and still is, incomparably the best drug of the group. Fortunately the physician is no

longer uncertain concerning its approximate activity in most cases.

The reader will understand just what digitalis means for the heart from the following considerations: the effect of the drug is to make the heart-beat slower, more regular and more powerful, thus increasing the cardiac output and improving the circulation. The short time-intervals between beats are the only period of "rest" allowed the heart. Now if a pulse of say 120 beats per minute is reduced to say 100 beats, that would mean a saving for the heart of 20 beats per minute or 28,800 beats per day. The brief periods of rest are thereby increased in length, and while still very brief, the total period of rest is thereby increased 20/120 of 24 hours, or no less than 4 hours! Not all of this, of course, is clear gain, as the beats are more powerful. But the net effect is of tremendous benefit to a laboring heart.

Active Principles of Digitalis.—The first fairly pure substance of the digitalis group was prepared about fifty years ago and is called crystalline digitaline, or, more commonly, digitoxin. It is an extraordinarily active substance and probably it would be used to the exclusion of digitalis were it not for the fact that it is impossible to prepare it of absolute uniformity except at prohibitive cost. The result is that some specimens are more active than others. Physicians have hesitated to use it partly on this account, partly because they have become accustomed to use digitalis and it is not easy to learn the complicated technic of employing a new member of the group with different periods between doses and differences in the adjustment of the dosage when once the effects begin to appear. This is one of those questions

that involve so many technical points that they cannot be discussed here in detail.

Schmiedeberg was able to prepare only two grams of pure digitoxin from ten thousand grams of digitalis. Even that small amount of the active principle was obtained at the cost of much time and skillful manipulation. Hence one can appreciate the great cost of this pure digitoxin. Enough has been prepared to permit of the study of its properties, and when more economical methods of preparation have been devised it is probable that it or another substance with nearly similar properties will replace digitalis and other crude drugs in the treatment of cardiac disease.

Every discovery of a method for the preparation of a pure principle of this type helps to a better understanding of the way to use these drugs. Such work must eventually lead to the employment of one of the pure principles in place of the many crude digitalis bodies now employed. That the pure principles hitherto discovered have not come up to the highest hopes merely means that we still have much to learn concerning the chemistry of these drugs. Every failure to accomplish the ends sought is offset by the fact that every discovery advances the quest nearer to the goal, and none of these discoveries has been without benefit in the solution of the larger question of the successful treatment of cardiac disease. Only a few days before these lines were written the representative of a firm of manufacturing chemists gave to the writer a small ampule containing a beautiful crystal of scillaren which his firm had obtained from squill, a drug that has been in use for more than two thousand years. Crystalline scillaren has some of the properties of digitoxin, but whether it will prove more

useful for oral administration in the treatment of cardiac disease remains to be determined.

Ouabain and Strophanthin.—Another pure principle of this group, known as ouabain and also as *crystalline* strophanthin, was discovered many years ago by Arnaud. It is used comparatively little in therapeutics because it is absorbed very irregularly from the gastro-intestinal tract, the consequence being that the oral dose cannot be adjusted satisfactorily. If a large dose is given serious poisoning may result, if less is given the amount absorbed may be insufficient to afford relief; there is no way of knowing how much will be absorbed in any case. It is injected intramuscularly or intravenously in cases of sudden heart failure, and many lives have been saved in this way that would have been lost.

Fraser prepared *amorphous* strophanthin from strophanthus seed, and much that has been stated about ouabain applies equally to strophanthin. It also is absorbed very irregularly after oral administration, hence it is practically useless in therapeutics except for intramuscular or intravenous injection. Every one who has been almost literally snatched from the jaws of death by the use of strophanthin during acute heart failure owes his life to chemistry, for the tincture of strophanthus is not nearly so useful. Not every one who has acute heart failure would die without ouabain or strophanthin, but the number of lives that have been saved in this way is large in the aggregate.

Cymarin is a pure principle prepared from apocynum or dogsbane. Its properties are nearly similar to those of strophanthin and it has many of the limitations of the latter.

While the pure principles of this group that have been isolated do not lend themselves to routine oral administration they serve an important use in studies of the many details of the actions of these substances. One is found to be more active in inducing constriction of blood vessels, another is more active in slowing the heart and still another is more actively emetic, and vomiting is one of the serious side actions of all members of the group without exception. A great many investigations have been directed toward finding a principle that would have the desired cardiac action without causing vomiting, but the principal lesson learned from such researches is that greater care must be observed in the regulation of the dosage, and that, of course, is additional evidence of the need of a pure principle that is suited in every way for oral administration.

Standardization.—Ouabain also serves a useful purpose in standardizing digitalis (biologically, with the aid of frogs), so that the physician is no longer under the necessity of using digitalis and other members of the group without knowing their activity after they enter the circulation. Unfortunately, he does not know the exact rate of absorption after oral administration, and there is urgent need of further research to the end that we may have a pure principle that will replace the crude drugs as completely as quinine and quinidine have replaced Peruvian bark in the treatment of malaria. We shall then wonder how physicians managed to get along as well as they do now without the pure substance.

It may be added here that digitalis preparations are used not only in the treatment of actual diseases of the heart but also in preparing the heart for inevitable strains in disease. Thus, a physician may employ it from the

very outset of a case of pneumonia to strengthen the heart for the extraordinary strain it faces. Since the effect of digitalis itself is slow, but persistent, caffeine is occasionally used for the first two or three days until the desired digitalis effect is evident. Because a fatal result in pneumonia is frequently due to heart failure, it is not surprising that this special care of the heart with the aid of digitalis may reduce the mortality. Similarly, digitalis is used before child-birth, to prepare the patient's heart for the extraordinary strain of labor if the clinical indications demand precautionary measures.

Accessory Drugs.—At rest and still more during sleep the needs of the body for fresh supplies of oxygen carried by the blood are, as stated before, appreciably reduced. There is a smaller demand on the circulation, and consequently the heart, although it must not miss its beats, is called upon for less work during rest and sleep. It is for this reason that in acute heart cases morphine is sometimes resorted to by a physician in order to induce sleep and bring rest to the heart.

Cardiac trouble may bring on a condition of edema (water), for instance, of the lungs, which in turn makes the work of the heart so much the heavier, creating a distinct menace to life. In this condition, atropine, the active principle of nightshade or belladonna, often brings quick relief, saving life.

We owe the discovery of all of these pure accessory principles, caffeine, morphine, atropine, to the skill of the chemist. It may be emphasized here, however, that in no other field of healing is the need of the *art of medicine* more imperative than in the treatment of acute heart conditions: there is no time to wait for laboratory findings—the experienced mind of the physician must

take in the whole picture at once and he must adjust his measures almost from moment to moment to what the picture means for him.

The drugs that are used in the treatment of cardiac disease have been discussed, and one usually thinks of chemistry as aiding the heart only through the discovery of new remedies. But modern medicine utilizes chemistry in its studies of the normal mechanism of the heart beat as well as that of the abnormal mechanism of cardiac disease, for it is impossible to know how to treat heart disease to the best advantage until we know wherein the diseased heart differs from the healthy one.

Influence of Potassium Salts.—It has been obvious from early times that the blood supply is essential for the normal functions of the heart, but it is only within recent times that we have come to understand the importance of extremely small amounts of certain salts of the blood and the influence exerted by even slight changes in its composition. Small amounts of potassium salts are essential for the heart beat, large amounts are poisonous. It has been found recently that under certain conditions the behavior of the heart toward potassium is an index of its behavior toward therapeutic doses of drugs of the digitalis group, and that those hearts which do not respond to potassium are incapable of benefiting by the use of digitalis. Such studies lead to a better understanding of the problems of diagnosis and treatment of cardiac disease.

Glucose and the Heart.—Insulin, the discovery of which has so recently stirred the whole of the civilized world because of its extraordinary value in the treatment of diabetes, is also capable of injuring the heart severely when the proper dose is somewhat exceeded. Formerly

there was no way of determining the exact amount of the active principle in insulin, hence there was no way of giving a sufficient dose without danger of giving too much. The unremitting labor of various chemists has resulted in producing insulin of far more nearly uniform potency than was possible at first, and Professor John J. Abel has recently succeeded in preparing crystalline insulin of an extraordinary degree of purity. The importance of this discovery can hardly be estimated. It is obvious that all diabetic patients who use insulin are under a great debt to chemistry.

Glucose, a sugar, is an essential constituent of the blood, and when it is present in amounts much below the normal the energy of the heart is impaired. Overdoses of insulin cause a great fall in the amount of glucose in the blood, but since the chemist has determined the normal amount it is comparatively easy to compensate for the loss by administering sugar or candy, or by the injection of glucose itself in urgent cases.

Epinephrine and the Heart.—During severe shock the circulation is insufficient and death may follow if the patient is not relieved. This condition is sometimes beyond human help, but not infrequently it depends on conditions of a temporary nature, and if this condition can be relieved the patient recovers. Professor Abel isolated the substance known as epinephrine or adrenaline from a gland of the body, and this substance has the property of causing a great increase in the strength of the heart beat and improvement in the circulation. Epinephrine has occasionally caused a heart that had stopped to beat again. Dr. Abel has also prepared a substance of great activity from the pituitary gland which has an action resembling that of epi-

nephrine, but the effects, while not so energetic, are more lasting. It has other important therapeutic effects which do not require discussion here, however.

It was stated before that every drug that enters the circulation exerts some action on the heart, pronounced in some cases, negligible in others. Every discovery of a drug which exerts its therapeutic effects with less of the undesired side action is of benefit to the heart. Hypnotics have come into more extensive use than formerly, whether it be that the strain of modern life necessitates the more frequent use of such remedies, or because people are more insistent in demanding relief for minor ills. The fact remains that the use of the hypnotics has increased greatly within the past ten or twenty years. Formerly, chloral hydrate was the most commonly used of the hypnotics, although it was known that it injures the heart unless it is used with great caution, and it is probable that it cannot be used continuously for any considerable period without some injury to the heart. Chemistry has supplied us with a great variety of hypnotics, indeed they appear faster than their exact therapeutic value can be appraised, but it is certain that many of those now available are less apt than chloral hydrate to injure the heart when they are used with caution. It is difficult to estimate to what extent many of us are indebted to chemistry in this field.

This is not a complete review of the benefits that chemistry has rendered to the heart, but it serves rather to suggest the extraordinary debt which we now owe, and the far greater one that we shall owe for its aid to the heart when many of the problems now undergoing intensive study shall have been solved. It would be a great mistake for one to suppose that because much

has been accomplished little remains to be done. All that has been done in this field is little more than a beginning, and it is fair to assume that chemistry will perform far greater services in the future because we are now just beginning to understand many of the fundamental problems of the heart in health and disease and those of treating it therapeutically.

Chapter VIII—Article 6

CHEMISTRY AND HIGH BLOOD PRESSURE

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Dr. Major's studies in European universities (Leipzig, Munich and Heidelberg) and in this country (Chicago and Johns Hopkins) prepared him for his successful career in medical research. His investigations have included important work on endocarditis, nephritis and diabetes, and other subjects, but he is best known for his recent striking work on the difficult problem of the causes of high blood pressure. Every advance in our knowledge of the causes of high blood pressure is of importance to millions of sufferers.

High blood pressure is one of the most common diseases of our time. While it is very difficult to obtain statistics regarding its prevalence, it is probably a conservative estimate that there are at least five million people in this country alone who have high blood pressure.

The number of people with high blood pressure is increasing, and one reason for this is quite obvious. The average expectancy of life in Geneva during the sixteenth century was 21 years, while in the United States in 1921 it was 58 years. High blood pressure is usually a disease of middle age and after, so that with the increase in the probable duration of life the number of people particularly susceptible increases. Because of this increased life span, we may expect an apparent increase in the diseases of later life.

This increase in the life span is not, however, the only cause for the increase in the number of hypertensives—as we term patients suffering from hypertension or high

blood pressure. There is little doubt that the people of the present century live under great stress and excitement. The exactions of modern life throw great strain upon the heart and the circulation, while the mode of life is not that which spares or strengthens the circulatory system. The increased pressure of daily life is, in many people, accompanied by an increased blood pressure, at first probably necessary just as an increased steam pressure is necessary for turning larger engines. This increased pressure in both man and in engines, however, produces greater wear and tear on the machine and shortens its period of activity.

The normal blood pressure is maintained by three factors—the force of the heart beat, the quantity and quality of the blood and the resistance the blood finds in the blood vessels. In many respects the circulatory system resembles a water-works system, with the heart as the pump, the blood taking the place of the water, and the arteries, veins and capillaries playing the rôle of the water mains and pipes. Unlike a water-works system, however, the heart is very sensitive to increased or decreased demand for power, the blood can rapidly change both its volume and its thickness or viscosity, and the arteries, veins and capillaries, instead of being a series of rigid tubes or pipes, are elastic structures which can readily dilate or contract.

While many of the conditions governing the circulation and blood pressure are purely physical or mechanical, like those in the water-works system, yet many factors largely chemical enter into the problems of the circulation, in health and in disease. These chemical factors, while making the whole subject more interesting and fascinating to the investigator, increase its com-

plexity and make it more difficult to understand, particularly because many chemical substances produce their effect upon the heart, the blood and the blood vessels, not singly but collectively.

Since one factor in the maintenance of the normal blood pressure is the force of the heart beat, it follows that any increase in the heart force may increase the pressure. Many chemical substances, such as caffeine, camphor and digitalis, increase the force of the heart beat and elevate the blood pressure, particularly when the heart is flagging and the blood pressure has fallen below normal.

The second factor in the production of high blood pressure is an increased volume of the blood and an increased viscosity or what we may term an increased thickness of the blood. The most striking cause of an increased viscosity is a rare disease, polycythemia, in which the red blood corpuscles are enormously increased in number, the red blood cells of such patients being often twice or more the number found in healthy persons. This second factor, while exerting its effect largely through mechanical means, is nevertheless profoundly influenced by chemical agents. The amount of fluid in the circulatory system may be increased by the presence of certain substances in the blood—sodium chloride and glucose, for example, draw fluid from the tissues and increase the blood volume. As long as the kidneys are functioning properly, this excess of fluid with its chemical substances is excreted rapidly and no elevation or, at most, a transitory elevation in blood pressure follows. Where the kidneys are diseased and unable to excrete properly, an elevation in blood pressure may result.

The most important factor, however, in the produc-

tion of high blood pressure is an increased resistance which the blood meets as it flows through the blood vessels. This increased resistance may be found in the arteries, the arterioles or smaller arteries, or in the capillaries, those smallest divisions of the blood vessels which connect the smallest arteries with the most minute veins. The capillaries form a vast network of small, ramifying vessels many miles in extent, a vast irrigation field, yet extremely close to the terminal arteries and veins, and, after all, a short space, as Osler said, "in which the real business of life is transacted."

The small arterioles and capillaries have the power of relaxation or dilatation and contraction or constriction, these changes being produced by the action of small nerves supplying the vessel walls—the vasomotor nerves. There is abundant evidence that most examples of continued high blood pressure are due to an extensive abnormal contraction of the arterioles and capillaries. The contraction of the capillaries has been observed microscopically in several conditions accompanied by increased blood pressure. The causes of this constriction of the capillaries are a very interesting problem which has been the object of extensive research for a generation. These investigations have by no means solved the problem, but they have brought us much information and also a growing conviction that the problem is largely one of chemistry.

Mental Factors.—One of the most frequent examples of constriction of the capillaries, with a resultant rise in blood pressure, is seen in fright and excitement. Many individuals, as the result of emotional strain, worries of business or of unhappy domestic relations, show a long continued elevation of blood pressure which falls

when the exciting emotional cause is removed. Many patients with certain types of mental disease, accompanied by great excitement, show an elevation of blood pressure which falls when the attack passes off and a clear mentality appears.

While such attacks of hypertension have usually, perhaps, been regarded solely as psychic, mental or "vitalistic" in origin, yet they may eventually prove to be intimately associated with disturbances in the metabolism of the body—in other words, a perversion of the chemistry of the body. What these changes are we do not know, nor can we scarcely hazard a guess. But we do know that mental states—fear and excitement—may cause other changes which can be detected. Fright easily produces a marked increase in the sugar of the blood in a rabbit, and excitement may increase the blood sugar in man. Students, during the stress and excitement of examinations, may secrete sugar in the urine, although they show none at other times. Such observations suggest that the mechanism of the increase of blood pressure in excitement may be, in last analysis, a chemical reaction.

Effects of Nephritis.—One of the most spectacular rises in blood pressure is that seen in acute Bright's disease or nephritis, which is produced by a well known bacterium, the streptococcus. In this disease, as the result of an infection, some substance is formed which causes a marked constriction of the arterioles and capillaries throughout the body, and a rise in blood pressure results. The same constriction of the small vessels takes place in the kidneys, the normal secretions are interfered with, bloody urine appears and the secretion of urine may be later entirely suppressed. The urinary dis-

turbances are so marked and often lead to such disastrous results that they have naturally focussed most attention upon the kidney, and the disease was long considered solely in the light of a kidney disease. More recent observations, however, seem to show that it is often a generalized disease of the smaller blood vessels and that the kidney disease is only a part of the picture. Microscopic study of the capillaries in the fingers of patients have shown the marked constriction taking place, and have made it possible for the physician to predict that a patient with a normal urine would soon show an acute nephritis with blood in the urine and an elevation in blood pressure.

The exact chemical substances producing this elevation of blood pressure are not known. It has, however, been shown that bacteria in growing produce substances, probably amines, which cause constriction of the muscles of blood vessels and may in that way cause an elevation in blood pressure. The further study of these chemical substances will doubtless make more clear their rôle in disease.

In chronic Bright's disease or chronic nephritis we usually encounter a marked and persistent elevation in blood pressure. In this disease the small vessels in various parts of the body are diseased, but the most striking changes are found in the kidneys and particularly in the glomeruli or filters of the kidneys. There are approximately 2,000,000 glomeruli in the human kidneys, and as the disease progresses these structures are gradually destroyed until the kidney is no longer able to filter the urine, and the disease becomes very serious.

The inability of the kidney to secrete the urine, which contains many of the waste products of the body, leads to an accumulation of these waste products in the blood, and they produce serious symptoms, the most marked of which is uremia. We do not yet know all of the substances that are increased in the blood of patients with chronic nephritis and uremia. Urea, creatinine, uric acid and sugar are known to be increased, but these substances are only a fraction of the chemical substances in the blood. It is highly probable that some substance or substances of a nitrogenous nature are increased, and that they are responsible for the elevation of blood pressure and serious symptoms—such as convulsions, seen in chronic nephritis and uremia. There is some evidence that guanidine* may be one of the chemical substances which plays a rôle. This substance, when injected into animals in small amounts, produces a marked elevation in blood pressure, while larger amounts produce convulsions and death. The definite proof that it is the poison responsible, however, has not been given.

Influence of Hormones.—The association of high blood pressure with disturbances of the glands of internal secretion, the so-called endocrine system, has long been the subject of study and speculation.

One class of the important glands of internal secretion are the adrenal glands, two small structures which lie just above the kidneys—one on either side. From these glands, a chemical principle or hormone, epinephrine or adrenaline, has been isolated, whose chemical structure

*Guanidine, $NH_2.C(:NH)NH_2$, is closely related to urea, $NH_2.CO.NH_2$, and to carbonic acid, HO.CO.OH. Like urea guanidine is a product of the chemical breakdown of nitrogenous food products in the body. Fortunately it is formed in much smaller quantities than urea.

is well known and which can be manufactured synthetically in the laboratory. Epinephrine, which is extensively employed in medicine, produces a marked rise in blood pressure when injected into the body.

Many investigators believe that under certain conditions an excessive secretion of epinephrine occurs and produces a rise in blood pressure which may be continuous. The proof of this theory is lacking, but it is of interest that injections of epinephrine produce an increase in the sugar of the blood and that many patients with high blood pressure show increased blood sugar. In Addison's disease, moreover, where there is a tubercular process of the adrenals, with presumably a decreased output of adrenaline, the patient shows a very low blood pressure, which is one of the striking features of the disease.

Women who have passed the menopause often have a very distressing train of symptoms—such as headache, dizziness and often hypertension. Fortunately, in most instances, these symptoms gradually abate and finally disappear. The hypertension which is often observed is usually benign and gradually subsides. These symptoms, which are so frequently seen, have been thought by most observers to be due to the absence of some internal secretion or hormone of the ovaries which is present when the ovaries are physiologically active and which disappears when the ovaries atrophy and cease functioning at the menopause.

The nature of these ovarian secretions is being very extensively studied at the present time. A substance has been isolated from the ovaries which has the power of

lowering the blood pressure, and it has been suggested that normally this substance antagonizes something which raises the blood pressure. When the ovaries cease secreting this substance, according to this theory, the blood pressure rises because of its absence.

These ideas still belong entirely to the realm of theory, but there is abundant evidence that the ovaries secrete certain substances, playing a very important physiological rôle. The exact nature of these substances is a fascinating problem for chemical study.

"Essential Hypertension" and Body Poisons.—In addition to these various types of hypertension, there is another group, perhaps the largest of all, which has been termed, for the want of a better designation, essential hypertension. People suffering from this type of hypertension show no Bright's disease, either acute or chronic, they are suffering from no great mental excitement or stress (although they are often "high strung" individuals), and they are not lacking in ovarian secretion, since many of them are men.

While the cause of this type of hypertension has not been established, there is a growing conviction that it is metabolic in origin, the result of disturbances in the normal chemical processes of the body.

A distinguished English physician of the past generation, Sir William Broadbent, writing nearly forty years ago on hypertension, in discussing what he considered the most important part of the mechanism producing an elevation of blood pressure, the capillary constriction, stated its cause "can scarcely be other than some substance present in the blood which acts directly upon the capillary walls." He also noted that "the special material which plays this part is almost certainly nitrogen-

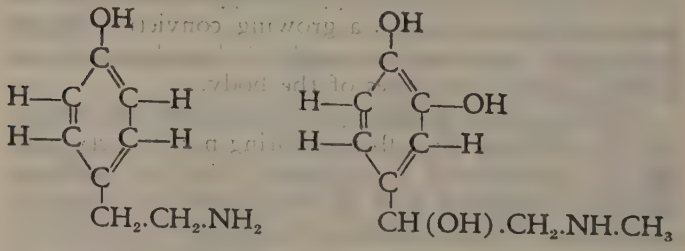
ized waste which has not undergone the complete oxidation necessary for elimination." He adds, "this is difficult to prove."

Later investigators will doubtless all agree with his statement that it is difficult to prove. It is as yet far from proved, but there is much suggestive evidence that indicates Broadbent was upon the right track.

Study of the blood of patients with hypertension frequently shows increased amounts of sugar, uric acid and cholesterol. These substances have no effect upon the blood pressure, but their presence in abnormal quantity suggests a disturbance of metabolism.

Recent investigations have shown that there are certain substances produced in the body which, when injected into animals, cause an elevation in blood pressure. One of these substances is tyramine.

Tyramine, or para-hydroxyphenyl-ethylamine, is chemically closely related to epinephrine, the secretion of the adrenal glands, as the following comparison of their chemical formulae shows:

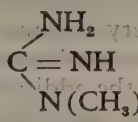


Tyramine Epinephrine

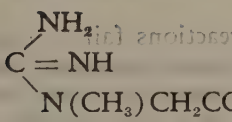
Tyramine occurs commonly in the products of putre-

faction, autolysis and fermentation, and must, therefore, be present in the alimentary tract.

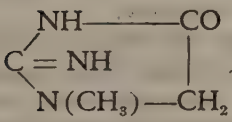
Another substance that has been under suspicion is guanidine. Methyl guanidine has been described in the urine and is apparently present as a kind of inner nucleus in many proteins. While there is some dispute regarding its presence in the body as a base or a salt, there is no question regarding the presence of its more complex compounds, and that they play an important rôle in metabolism. The relationship of some of these substances to guanidine is very close.



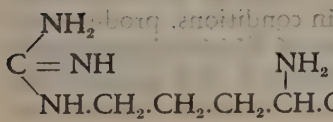
Methyl Guanidine



Creatine (Methyl guanidine acetic acid)



Creatinine (Creatine anhydride)



Arginine (Guanidine amino valeric acid)

Arginine is formed in the intestines during the process of digestion and is regularly absorbed by the blood vessels draining this area. Creatine is present in all of the skeletal muscles in large amounts and plays some important part in the nutrition of the muscle. Creatinine is present in the blood and is excreted in the urine. None of these substances when injected into animals produces any change in blood pressure. Methyl guanidine, on the other hand, when injected in small doses, produces a marked and relatively lasting elevation in blood pressure and, in larger doses, convulsions and death.

These differences between the action of methyl guanidine and its derivatives are very striking. While methyl guanidine itself in relatively small doses is poisonous and raises the blood pressure, the addition of acetic acid, with the formation of methyl guanidine-acetic acid, or creatine, produces a substance which is not a poison, but is necessary for life.

If these normal chemical reactions fail to take place, it is easy to see how methyl guanidine might fail to be converted into creatine, accumulate in excess and produce an elevation in pressure. It is also possible that the kidneys may excrete these substances improperly and thus give rise to an excess in the body.

It must be emphasized that the rôle of tyramine or guanidine in the production of high blood pressure has not been definitely proved, although both are under suspicion. The same may be said for other substances, particularly the products of bacterial decomposition. Bacteria, growing under certain conditions, produce substances, probably amines, some of which raise the blood pressure and some of which lower it. It is possible that such bacterial substances may be absorbed from the in-

testinal tract and elevate the blood pressure, and this same absorption is possible in the case of tyramine and methyl guanidine.

A survey of our precise knowledge regarding the cause of high blood pressure shows that we have perhaps accumulated in our search more theories than demonstrated facts. The value of the work in this field is, however, very great, and there is excellent evidence that chemical factors are very largely responsible. The search for the cause of high blood pressure continues, and we may confidently hope that all the work of the past, although it contains much error, also contains the grain of truth that will lead us on to an unraveling of the mysteries of this disease, one of the greatest scourges of the human race.

Chapter VIII—Article 7

CHEMISTRY AND THE KIDNEYS

EDWARD J. STIEGLITZ, M.S., M.D.

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Dr. Edward J. Stieglitz, as clinical instructor of medicine at Rush Medical College of the University of Chicago, has had charge, since 1923, of the renal and vascular clinic of the Central Free Dispensary of the College. He is also on the Medical Service Staff of the Chicago Lying-In Hospital. He has published a number of notable papers, especially on chemical studies of kidney functions. Perhaps the most important one showed that the reaction (acid or basic) of the kidney tissues is the reverse of the reaction of the urine secreted, which serves as a warning against excessive alkalinization of patients.

Knowledge is strength but wisdom is power, or strength put to work. One of the most baffling questions of medicine has been the problem of disturbances of the kidney. It is only in recent years that rapid progress has been made, largely with the aid of the efforts and researches of the chemist. Any proper or thorough understanding of disease is dependent upon an accurate understanding of the normal mechanisms involved. Similarly, efficient ways and means of combating these disturbances are dependent both upon a clear conception of the actual changes which have taken place and also the cause thereof. Three centuries of continuous investigation have been necessary to reveal clearly the complex anatomical structure of the kidneys. The problem of how the kidneys as a mechanism perform their work is still partly unsettled, but in the last thirty years strides of greatest importance have been made, primarily by studies directed along chem-

ical lines. Anatomic and pathologic investigation has merely formed the groundwork upon which the chemical researches into kidney function have been based.

THE FUNCTIONS OF THE KIDNEY

In brief, the functions of the kidney, or the work which it normally performs, may be grouped into four divisions. The first of these is the elimination from the body of waste water, or water in excess of the requirements of the tissues. Secondly, the kidneys are the chief means of eliminating excessive quantities of salts of all kinds. Without this continuous excretion of salts, the constant intake in food and drink and the creation of salts in the tissues as a result of tearing down food molecules, would rapidly lead to an accumulation in the body, which would greatly alter the chemical conditions and result within a short time in death. The normal limits of balance of salts, both in the blood and tissues, are very close, with only small variations permissible in health. The kidneys, therefore, may be regarded as of similar function as the parasol of a tightrope walker: they are essential in maintaining balance or equilibrium upon a narrow path. The third function of the kidney is that of elimination of waste products of the body other than salts, particularly the products containing nitrogen. The fourth important process carried out by the kidneys is their work in assisting the body to maintain not only the balance as regards salts in general but also the balance of the various individual salts and particularly those which determine the balance of acid and alkali in the blood and tissues. The neutrality of the blood and most of the tissues is rigidly

maintained by the normal kidneys and what fluctuations have been observed are of a most minute degree. No matter whether considerable amounts of alkali, as for example sodium bicarbonate, or acid substances, are ingested, the kidneys promptly rid the body of excesses in either direction and thus maintain the constant equilibrium. Daily, hourly, even every minute, forces are at work tending to destroy this delicate balance, but the vigilance of the kidney is unceasing.

The mechanism by which these activities are carried out represents one of the most complex and difficult problems confronting the science of medicine today. Much has been accomplished but more remains to be done. The probable mechanism is that the cells of the kidney do not act as a simple semi-permeable membrane as was once presumed, but are actively engaged in withdrawing from the blood the excess salts, water and other waste, and thereby forming and secreting the urine. The urine is often more concentrated as regards certain elements than is the blood, so that the kidney apparently exercises what might be regarded as a fine discrimination not only as to what to remove from the blood but also as to how much. That this process entails a large amount of work is indicated by the great consumption of oxygen from the blood by the kidneys. The functions are even more complex, for the kidney does more than merely pass on in altered combinations those waste products delivered to it by the circulating blood. For example, should there be an excess of acid to be removed, the kidneys manufacture ammonia from urea to assist in neutralizing the excess of acid. This important activity of the kidney was beautifully demonstrated by Nash and Benedict by chemical methods.

The kidney is also particularly active in eliminating substances foreign to the normal blood, such as poisons, and therefore belongs to the group of organs which act as defensive structures to the delicate human machine.

How have these briefly outlined facts and conclusions anent the mechanism of kidney secretion been obtained? Largely through studies fundamentally chemical in nature. Accurate quantitative determinations of the amount and nature of the salts and other constituents of urine are dependent upon the development of methods of quantitative chemical analysis. Facts concerning the amount of salt or nitrogenous waste removed from the blood in a given period of time and under various controlled and known conditions have been of utmost value in determining the exact mechanisms involved. A second method of attack upon this problem has been that of studying the progress of specific substances, detected by means of chemical reactions whose products are made visible within the actual cells of the kidneys under the microscope. This mode of investigation may be well termed "microchemical." Among these investigations may be mentioned those dealing with the kidney secretion of iron which were made possible only by the chemist's contribution of an extremely delicate test for iron, which reveals the iron in the form of easily visible deep blue particles (Prussian blue) even in very minute quantities. Of clinical significance are similar studies showing that in pernicious anemia such deposits of iron through accumulation cause injury to the kidneys.

Illuminating studies dealing with the methods by which the kidney assists in controlling the acid-alkali balance of the body have recently been carried out by

the use of sensitive "indicator" dyes. These dyes have the property of rapidly changing their color in the presence of either an acid or an alkaline medium. Thus it is possible to use the dyes to determine whether a previously colorless cell or fluid is either acid or alkaline in reaction. The introduction of such dyes into the living kidney has made possible accurate studies of the rôles played by different structural elements in this important activity.

The physical chemists, through their rapid advancement of our knowledge of the effects and laws of membranes and surfaces, have tremendously advanced the opportunity for study of the mechanism of urine secretion. The reactions of various elements when mixed together in a simple solution are quite another matter than the reactions of these same elements when present on different sides of a membrane which permits only certain of the molecules to pass through and bars others. Similarly, colloid or gelatinous mixtures in which minute droplets or particles of one medium, as for example oil, are suspended in water or *vice versa*, are entirely different in behavior from simple solutions. The living cell, of the kidney or elsewhere, consists of just such colloids, containing many thousand different substances in complex combinations, separated by surfaces and limits. For example, in an attempt to understand the rôle of iodine in cell life and cell activity, it does not suffice to know that there is iodine within the cell. Chemical methods of microscopic study have shown that parts of the cell may be quite free of iodine, whereas other parts, in close proximity, contain considerable amounts.

It becomes more and more obvious therefore that the

solution of the fundamental problems of cell life in general and of the kidney in particular are dependent upon the assistance of chemistry and physics. New methods of analysis and synthesis, new facts and basic physical laws, and new conceptions and theories are all of inestimable value and necessary for the final solution of this complex but essential problem. With the newer knowledge, surer methods to aid patients are bound to follow.

CHEMICAL CAUSES OF KIDNEY DISTURBANCES

The above discussion has served to illustrate a few of the many factors involved in a critical study of the normal mechanism of kidney function. Chemistry is of equal assistance in illuminating the causes of renal injury. Not only are the chemistry and mechanisms of known kidney poisons being explained, but constant investigation has revealed many new and unsuspected sources of kidney damage. A few examples of the chemical factors in the causation of kidney injury will suffice to illustrate this point.

Excess of Meat in Diet.—For many years an excess of meat or other protein food in the diet has been considered a cause of kidney damage. Recent work from the University of Michigan has demonstrated that only a certain few of the more complex amino acids in such foods are injurious; among these, histidine, tyrosine and tryptophan may be mentioned. Others are quite harmless even in liberal quantities. Very small amounts of the injurious amino acids are sufficient to cause considerable damage to the kidneys. Investigations are now in progress to determine why this variation in toxicity

exists and if any chemical means are available for the neutralization of such poisonous effects.

Metal Poisons and Antidotes.—Mercury and its compounds are extremely poisonous to the kidney even in very minute amounts. To the average reader this fact may seem of relatively minor significance because of a failure to appreciate the frequency of contact with this metal and its derivatives. Severe and even fatal poisonings are often the results of accidental or deliberately suicidal ingestion of mercuric bichloride, or a consequence of faith in the purity of bootleg liquor made with alcohol denatured with this violent poison. Similarly lead, arsenic and others of the heavy metals are particularly injurious to the kidneys, primarily because of the fact that the kidneys attempt to rid the body of these poisons and through accumulation are exposed to greater concentrations than are other tissues. Lead is a frequent hazard, both industrially, as for example from lead dust from dry paints, printer's type, cable sheathing, heavily leaded paper, and also incidentally from leaded silk in hose and from lead water pipes. While such pipes in houses are to be found now only in very old buildings, few people are aware that lead pipes are still in use in many cities to connect the water mains in streets with the pipes serving the houses. The prevalent widespread use of chlorine in the water supply of cities increases the risk of lead contamination of water from this source, because chlorine attacks lead more readily than does pure water. In a recent investigation by the U. S. Public Health Service of the lead hazard associated with the use of lead tetraethyl in "ethyl gasoline" one of the most surprising results obtained was the evidence of the daily excretion of

very small but definite amounts of lead by the average city resident. In themselves these amounts were within the range of tolerance, but the observations showed unquestionably that we are that much closer to the threshold beyond which chronic poisoning must result. Any such poisoning would involve the kidneys. Arsenic forms the active principle of several of our most valuable drugs. Physicians are ever alert to detect evidence of kidney injury during and after the administration of arsenical drugs.

The chemist, however, besides creating many of these forms of menace to health in our daily lives, has now also found a remarkably efficient antidote for the heavy metal poisonings in sodium thiosulphate, the ordinary "hypo" of the photographer. If administered promptly, either by mouth or directly into the blood stream from the sterile ampoule solutions now available, this salt has an extraordinary effect in combining with the metal and rendering it inert, exactly as "hypo" combines with and dissolves the excess of silver on a photographic film or plate and thus "fixes" it.

In actual clinical practice, chemistry is often of immeasurable value in differentiating between two or more possible causes of damage, and thereby determining logical treatment. It may be illuminating to relate one such instance. A young woman, approximately six months pregnant, was admitted to the hospital acutely ill with bloody urine, marked dropsy, murmuring incoherence of speech and involuntary twitching of the muscles. She had symptoms of severe kidney injury, such as may occur suddenly as a result of the poisoning of pregnancy (eclampsia), and the question arose whether as a life-saving measure the pregnancy should

be terminated. Questioning of the family, however, revealed that several days before, feeling very well, the patient had re-enameled some furniture, and had gotten considerable of the paint upon herself. This she proceeded to scrub off with turpentine. Now turpentine is easily absorbed through the skin and is very injurious to the kidneys. Hence, when chemical examination of the urine revealed the presence of turpentine, the cause of the renal injury was made clear. As a result a needless operation was avoided and the young woman became a happy mother.

Infection.—The most common cause of injury to the kidneys is infection. Infection is carried to the kidney by the blood stream from other parts of the body. Such infections of the kidneys are all too frequently sequelæ of acute tonsillitis, sinus infection, so-called "simple colds," scarlet fever, diphtheria and a host of other sources. How can the chemist help here? His work along these lines has barely started but already wonders have been performed. He has created synthetically new substances, deliberately planned to destroy the bacteria or to inhibit their multiplying but to leave the human host uninjured. To mention but a few of the outstanding internal antiseptic achievements we must include acriflavine, gentian violet, mercurochrome and the most recent contribution, hexyl-resorcinol, which is almost fifty times as powerful as carbolic acid in destroying bacteria and essentially non-injurious to the host. Two of these substances, mercurochrome and hexyl-resorcinol, are creations of American chemists at Johns Hopkins University. Hexyl-resorcinol is excreted by the kidneys in part unchanged and offers the possibility of obtaining a continuous flow of bactericidal

urine. It is effective against certain infections but not against all and must be used with discrimination. These contributions of chemistry have given medicine new and powerful weapons to combat infection of the kidney and the bladder.

THE CHEMISTRY OF KIDNEY FAILURE

Failure to Secrete Water.—To the non-medical reader the most common symptom of kidney injury is dropsy or edema. The puffy, waxy pale, swollen character of the skin and other tissues is due to an excessive accumulation of water in the body. Until twenty years ago it was assumed that this accumulation of water was entirely due to failure of the kidneys to rid the body of water and all sorts of efforts were made to stimulate the secretion of urine by the kidneys. We now know that the edema is not entirely due to changes in the kidneys but to changes in the body tissues in general, in the skin and other structures. This new point of view resulted from the introduction of chemical methods of study into the problem of water balance. Dr. Martin Fischer of Cincinnati and Professor Jacques Loeb demonstrated that with slight chemical changes in the medium bathing living cells, the cells could be made to swell with water or shrink and give up water at the will of the experimenter. Dr. Fischer studied not only the living cells, but simpler colloid mixtures such as gelatin, and was able to show that increases in acidity and of certain salts caused the gel to give up water and therefore to shrink in size. In the body swollen with edema exactly similar phenomena occur. It is the "thirst" of the chemically altered tissues that absorbs and binds the water and the reason for the small urinary

output is, in large part, that there is no, or very little, water available for secretion by the kidneys. The factors causing these changes in the tissues are now being investigated by chemical methods. Drs. McClure and Aldrich, Andrews and many others are rapidly advancing our knowledge in this field.

In this connection one particular phase is of special interest. Fischer and others contended that the swelling results from increased acidity in the tissues, so that their treatment consisted in the liberal administration of alkalies. The treatment is often effective, but inasmuch as the reaction of the kidney cells has been shown by the indicator method to be the opposite to the reaction of the urine eliminated, excessive alkali treatment is liable to cause injury to the kidney by increasing the acidity of the kidney cells and thus occasionally lead to suppression of diuresis, aggravating the dropsy. More recent work has demonstrated that certain acid-producing substances like calcium chloride give rise to a prompt and liberal flow of acid urine and a diminution in the edema. If the above explanation is correct, improvement by this treatment is probably due to favorable action on the kidney itself, the acidity of whose cells is diminished.

It is common medical and lay knowledge that caffeine, the active principle of tea or coffee, is a stimulant to the kidney, but it is also a strong stimulant to the brain and heart. Now, caffeine, chemically, is *tri*methyl xanthine. Chemistry has discovered two *di*methylxanthines, known as theobromine and theophylline, both of which surpass caffeine in diuretic power. Neither produces the mental excitement resulting from larger doses of caffeine. As a result, the physician can now secure

the diuretic effect alone. Theobromine is obtained from cacao and is also made synthetically; theophylline is found in very small amounts in tea and is prepared synthetically for medical use. Theophylline is the most effective and prompt diuretic but its effects are not as lasting as those of theobromine and it is sometimes irritating to the stomach and kidney. Theobromine is therefore usually preferred by the physician, sometimes preceded for a few days by theophylline. Chemistry has thus given to medicine a variety of specific aids for the treatment of edema, which have been of greatest benefit to thousands of chronic sufferers.

Furthermore, cooperation of the physician and the chemist has brought into use a new diuretic, novasurol, a complex salt of mercury and barbital, which was originally introduced as an antisyphilitic but is now chiefly used as a diuretic of even greater potency than the xanthine derivatives in certain types of edema, particularly those associated with heart disease. If used with acid substances as mentioned above, it greatly enhances their value. The presence of mercury in this compound limits its use in cases with marked kidney injury.

Failure of the Kidneys to Eliminate Salts and Waste Products.—Since the first accurate description of nephritis by Richard Bright early in the nineteenth century, medical men have realized that of equal if not greater importance than the accumulation of water in the tissues is the accumulation of waste products in the blood. When chemical methods of quantitative determination especially of nitrogenous substances of this character in the blood were devised it was discovered that high concentrations of urea, uric acid and other substances might occur without much evidence of disturbance of the

water balance, but with symptoms of poisoning of the brain, such as twitchings, convulsions, drowsiness, irritability or coma. For many years these symptoms were attributed to a direct poisoning of the nervous system by these retained waste products; hence the term "uremia." More recent and more exact clinical and experimental chemical studies, made possible by the important contributions of Dr. Otto Folin and his associates in devising methods of accurate analyses with small samples of blood, have demonstrated that these waste products are not directly responsible for the nervous changes. For the purpose of diagnosis and to enable a physician to clarify his conception of the gravity of a given situation and thus be in a position to predict the probable outcome of a kidney disorder, these chemical analyses of the blood are of greatest importance.

With their aid we know now that much more emphasis must be placed upon the rôle played by inorganic salts and faulty elimination of such salts by the kidneys in causing the symptoms of so-called "uremia." The effects of salts upon the condition and reactions of colloidal gels are far reaching. Sodium and calcium ions have distinctly antagonistic activity in their effects upon membranes and surfaces of gels and much of the change occurring in uremia may be attributed to a disproportion between these two salts. Aside from changes in the nervous system, such chemical disorders are to a great degree responsible also for abnormal contractions of blood vessels (spasms or cramps) which in turn cause abnormal elevations in blood pressure (page 511). Chloride ions in excess are reputed to be vascular irritants. For many years treatment of these conditions with small amounts of bromides or iodides has been

advocated, but it is only recently that chemical investigation has shown that under such conditions the iodide or bromide tends to replace the disturbing excess of chloride and thus assists in the restitution of normal conditions.

Even more recent have been the studies of the rôle of nitrate and nitrite ions in relaxing vascular spasticity, with the result that a new and rather effective method of attack against the insidious inroads of high blood pressure has been given to man.

Cooperation of medicine and chemistry has made possible the introduction by Rowntree of the dye, phenolsulphonephthalein, for the more accurate estimation of kidney efficiency in patients afflicted with injured kidneys. The dye is non-injurious and is specifically secreted by the kidneys. By experience it has been shown that if a known amount of the dye is injected, within two or three hours fifty to eighty per cent should have been secreted into the urine, where the amount may be quickly estimated. The use of this test of kidney efficiency has proven of particular value in cases where rapidity of procedure is important, as for example as a check upon kidney conditions prior to surgical operations.

Failure to Secrete Uric Acid: Gout.—The results of failure of the kidney properly to eliminate salts and waste products from the body are so diverse and complex both medically and chemically that any attempt at thorough discussion is beyond the scope of this article. But gout represents one particularly interesting phase of the subject both on account of its wide incidence and also because it illustrates well the value of cooperation between medicine and chemistry. Gout is

less frequent in America than in Europe. For many years it has been attributed to rich diets, containing excesses of meats and alcoholic beverages, particularly wines and beer. The influence of diet was strikingly shown in Europe during the recent war, for during the period of food restriction and control gout practically disappeared from the continental clinics, only to return after the war. Heredity also plays an important rôle.

Characteristic of gout are deposits of uric acid and urates, such as sodium urate, in the tissues. That deposits of some white crystalline substance occurred has been known from ancient times, but it is relatively recently that the presence of uric acid and its salts in this substance has been recognized. The chief source of uric acid in man is nucleinic acid, a complex chemical substance found chiefly in animal tissues rich in cells, such as the pancreas (sweet-breads), liver, brain and spleen. A diet of sweet-breads may indeed be used deliberately to bring on an attack of gout, and thus serve as a clinical confirmation of a diagnosis of gout. Nucleinic acid is also manufactured in the human body as in the bodies of other animals, as is demonstrated by its liberal excretion in the urine of infants receiving nothing but milk as food. The normal content of uric acid in the blood in health remains quite constant at from 2.5 to 3.5 milligrams per 100 cc. of blood.

Many years ago it was suggested that the accumulation of uric acid in the blood and tissues was due to a faulty elimination of this substance by the kidneys, rather than to an excessive production of uric acid, and recent studies have confirmed this. In gout the blood uric acid may rise to 3 to 10 milligrams per 100 cc. The contribution of biologic chemistry in discovering

a method for determining simply and quickly the exact concentration of uric acid in the blood, using only a small amount of the latter for the analysis, has proven of tremendous value and is of daily application as a means of differentiating between true gout and diseases presenting similar clinical phenomena. Moreover, gout is frequently associated with other chronic disorders such as angina pectoris, diabetes, high blood pressure. and kidney disturbances, eczema, hives, etc. The chemical analysis of the blood is thus of great assistance in clarifying the picture in each individual problem. It may easily be demonstrated that the undue accumulations of uric acid in the tissues are very irritating and produce experimentally the inflammatory reactions as seen in gout. The most frequent location of such uric acid deposits is about the cartilage which lines the joint surfaces. The irritation of these accumulations leads to the death and destruction of the cartilage with subsequent denudation of the underlying bone. The acute pain in the joints, most frequently in the great toe, results from irritation of the nerve fibers by the deposit.

A Chemical Remedy for Gout.—Not only has chemistry contributed thus largely to our more accurate understanding of gout and by means of chemical analysis of the blood made possible a more accurate diagnosis and a controlled treatment, but it has also created for the gouty an efficient remedy. Traditionally, colchicum has been used for years, but the newer synthetic cinchophen, introduced as atophan, is far more effective. The drug is rather bitter and occasionally produces unpleasant reactions in some patients. Creative chemistry therefore proceeded to improve upon it and created neocinchophen (novatophan) which is

tasteless, devoid of any unpleasant reactions and just as effective. Cinchophen and its derivatives stimulate the kidney so as to have a selective action upon the secretion of uric acid. The uric acid content of the blood is often reduced to half the pre-existing level by the administration of the drug. Similarly, the uric acid deposits in the tissues, or tophi as they are called, grow smaller and there is prompt relief from the severe pain associated with acute gout. The profuse output of uric acid into the urine as a result of such medication may in some instances be responsible for the formation of small stones or calculi, for uric acid and its acid salts are but sparingly soluble. The salts formed by complete neutralization of uric acid are much more soluble, so that this unpleasant complication may be avoided by the simultaneous liberal administration of sodium bicarbonate.

It must be kept in mind that valuable as cinchophen and neocinchophen are in the treatment of gout, they do not remove the cause of the uric acid accumulation as found in gout and that therefore dietary restrictions have a fundamentally important place in its treatment.

In this discussion of the problems of medicine dealing with disturbances of the kidney we have seen what notable progress has been made with the aid of all the branches of chemistry, analytical, physical and synthetic organic chemistry. There is still very much to be done. There is no unanimity of opinion even in regard to the precise mechanism of the fundamental processes of secretion by the kidney. The possibilities of preventing injury to the kidney by poisons of bacterial origin or from external sources need to be studied more exhaustively as well as the possibilities of securing chemical

and physical conditions for the rehabilitation of an injured kidney. Even with all the advances made, the almost infinite variability of conditions in disease teaches the wisdom of preserving medicine as an art, even while it is becoming more exact and scientific. No two patients with nephritis (or any other disease) present identical problems or identical reactions. The almost intuitive summing up in diagnosis by a physician feeling his way carefully among a variety of possibilities is based after all on an integration of all his knowledge and experience bearing on the evidence in a given case. This is characteristic of medicine as an art. Obviously, the more precise and the more complete the testimony rendered by the findings in the laboratory, the more unerring will this summing up be and the more effective will be the safeguarding of the health and life of the patient. Science progresses cautiously, one step at a time, carefully testing its present foothold before attempting further advance, as the Alpine climber makes sure of his footing before ascending further.

CHAPTER IX

The War on Invading Germs

ARTICLE 1

THE GERM THEORY OF DISEASE

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IT is not a matter of indifference what theories we hold respecting ourselves and the world around us. Inevitably our lives and our daily actions are swayed by what we believe and what we imagine. In no department of human life has this been more completely and in some ways more terribly demonstrated than in the changing opinions of mankind about the nature and cause of disease. The belief, for example, that in certain maladies and in certain bodily conditions there is too much blood in the body and that the excess of blood must be removed stains a tragic page in human history. No one can ever know what suffering was caused, or can attempt to estimate the countless lives

sacrificed to the practice of bleeding, once so common. When Alexis St. Martin, the Canadian voyageur made famous by the researches of Beaumont on digestion, received a gunshot wound, blowing off the left side of his body and exposing the stomach, it might be supposed that he had lost enough blood, but we read that within 24 hours "he was bled to the amount of eighteen or twenty ounces." So a dominant theory often induces man to cast aside all practical considerations. The belief that evil spirits sometimes "take possession" of the human body determines the way in which patients are treated among all savage peoples and has influenced the remedial measures applied to many forms of mental disease in highly civilized communities even in modern times.

The germ theory of disease must naturally take its chance along with the "humoral" theory, the spirit or "animistic" theory, the homeopathic theory and numerous other would-be explanations of disease causation. A theory can be justified only by the extent to which it accounts for the observed facts and by its success in enabling man to devise methods of prevention and cure. How does the germ theory meet these requirements?

It may first be asked, just what does the germ theory hold? As commonly understood today the germ theory maintains that infectious diseases are due to certain small microscopic plants or animals known collectively as microbes. We shall see that in respect to some of the most serious and widespread maladies with which man is afflicted, it is a theory no longer.

In a sense, the germ theory of disease originated from the germ theory of fermentation. Far earlier than this

it had been conjectured that disease might be due to minute living organisms. Writers in the Middle Ages and even early Greek philosophers had speculated about such possibilities, but their musings had had little effect on the current of human thought and none on the practice of medicine. The gap between wondering if a thing may be so and finding out whether it is so is a wide one as every experimenter knows. It was not, in fact, until the third quarter of the 19th century that an adequate experimental basis for the germ theory of disease was finally laid. When it became known through the work of Pasteur and others that the processes of fermentation, putrefaction and decay were not, as many had believed them to be, the effect of direct atmospheric oxidation but were due to the activities of living microbes, then the belief that certain diseases were also caused by microbes gained new force and became the subject of active experimentation. The rapid growth of understanding about the fundamental resemblances of fermentation and disease, the realization of the importance of the doctrine of spontaneous generation and the ingenious character of the technical methods used in studying these problems make one of the most fascinating chapters in the history of scientific discovery, but there is no space for their proper consideration here. We are limited for our primary discussion to the germ theory of disease itself.

There are still people who want to be shown the evidence on which the germ theory is based. Last year after I had read before a scientific association a paper on Influenza in which I dwelt on our lack of knowledge of this disease, I received a number of letters from that apparently large body of persons who are always

ready to explain what others do not understand. One of my correspondents, after expressing his own faith that "diseas is not given by A sell or nucleus," challenged me to a public debate on the cause of disease. It is always a satisfaction to give reason for the faith that is in us and to set forth once more the evidence on which we depend.

Diphtheria.—Why, for instance, do we believe that diphtheria is caused by a specific microbe? (1) When a child is ill with diphtheria it is well known that the seat of the disease is in the throat. Not only is there redness and pain, but these common signs of throat inflammation are associated with the formation of a kind of membrane which makes swallowing difficult and may extend into and clog the windpipe, causing suffocation. This membrane has long been known to be characteristic of diphtheria; in fact it gives its name to the disease. One might expect therefore that if a microbe were the cause of diphtheria it would be found with especial abundance in the diphtheritic throat and membrane. As a matter of fact microscopic examination of the membrane shows the presence in great numbers of a characteristic "beaded" or "barred" bacillus or rod-like organism which is not ordinarily found in the healthy human throat or in diseases such as whooping-cough, tuberculosis or simple tonsillitis. This bacillus, at first named from its discoverers the Klebs-Löffler bacillus, is sometimes found in healthy persons who have been in contact with diphtheria patients or in children recently convalescent from the disease. In many persons, too, microbes superficially resembling the Klebs-Löffler bacillus, the so-called diphtheroids, are present, but an experienced observer is generally able to

distinguish them quite readily from the bacilli found in diphtheria. There is therefore a characteristic microbe invariably present in the characteristic membrane of diphtheria and found elsewhere only under conditions where as a rule its presence can be readily interpreted. (2) Although the Klebs-Löffler bacillus does not occur in nature except in the human body, it can be artificially grown in a nutrient medium composed of peptone-beef-broth, glucose and serum. It is even possible by expert manipulation to separate a single living cell from its associates and from all extraneous material, introduce it into the culture medium and watch it grow. After some growth has occurred, a sterile needle may be dipped into the tube of culture medium and a transfer made to a fresh tube. At the end of a series of transfers only the remote descendants of the original bacilli can be present. Now if these Klebs-Löffler bacilli in pure culture be injected into a susceptible animal such as a guinea pig they will reproduce many of the typical symptoms of diphtheria. One instance is known in which a bacteriologist accidently drew into his mouth some of a living broth culture; although he spat the material out he developed a diphtheritic membrane on both tonsils with typical symptoms. There are also records of intentional self-inoculation and the inoculation of volunteers. In these instances true diphtheria with its attendant train of symptoms has been produced by contact with pure cultures of the Klebs-Löffler bacillus. (3) In the bodies of children who have died from diphtheria certain changes have been observed in the organs, and, with the aid of the microscope, in the tissues. The most seriously damaged organ appears to be the heart. Guinea pigs and rabbits inoculated with

the Klebs-Löffler bacillus show similar heart lesions; in guinea pigs there is also conspicuous swelling and reddening of the adrenal glands and while these organs are not so strikingly altered in human diphtheria, there is an accumulation of evidence that they are severely affected. There is, in a word, a general correspondence between the pathological changes in the human body after death from diphtheria and the changes in the bodies of animals that have been inoculated with the Klebs-Löffler bacillus. (4) Up to this point we have been considering only the possible connection of the living organism with the disease process. A few years, however, after the discovery of the Klebs-Löffler bacillus in the diphtheritic membrane it was found that a highly poisonous substance, the so-called diphtheria toxin, was present in broth cultures of the bacillus and that this substance, when freed by filtration or other means from the living organisms that produced it, could by itself cause some of the most characteristic symptoms and lesions of diphtheria. Guinea pigs injected with the germ-free toxic filtrate of the Klebs-Löffler bacillus succumbed in the same way and with the same changes in the heart and the adrenal glands as did animals inoculated with the living organism. The diphtheria toxin is of almost incredible potency. A teaspoonful of a broth culture of the Klebs-Löffler bacillus may contain enough toxin to kill more than 5000 guinea pigs. The toxin may be precipitated out of the broth and obtained in a more or less impure condition in the form of a yellowish gray powder. Nothing is known about its chemical composition except that it is closely associated with protein substances and may be a protein itself. (5) The

crowning achievement in the bacteriology of diphtheria is the discovery that animals injected at first with small non-fatal doses of diphtheria toxin and later with gradually increased amounts come to contain in their blood a substance capable of neutralizing the diphtheria toxin—the so-called diphtheria antitoxin discovered by Behrend. When experimental animals, after they had been inoculated with many times the fatal dose of toxin, were given antitoxin (obtained from the blood serum of immunized animals) they would miraculously recover. A dose of toxin sufficient to kill a thousand guinea pigs could be safely injected into one animal provided it was mixed with a neutralizing dose of antitoxin serum. The brilliant success obtained by the application of these experimental findings to the cure of human diphtheria is a matter of common knowledge. At this point it is sufficient to call attention to the additional evidence as to the causal significance of the Klebs-Löffler bacillus that is furnished by the use of antitoxin. Our belief is justified by the pragmatic test. The proof of the pudding is in the eating. The germ-free filtrate of a broth culture of the Klebs-Löffler bacillus injected into the horse gives rise to a substance in the body of the horse which before was not there—or not there in such abundance—and which in practice has proved a specific remedy for diphtheria. (6) More recently another piece of evidence has come to light. If an exceedingly minute quantity of diphtheria toxin be injected into the skin of the forearm, a slight reddening will be produced at the point of injection in children who are susceptible to the disease, while in children who have had diphtheria no such reddening occurs.

This skin reaction, the Schick test, has proved of practical value in determining which children are resistant and which are susceptible and need protection. It also obviously affords another bit of evidence as to causal connection.

There is thus ample reason for believing the Klebs-Löffler bacillus to be the true diphtheria bacillus and the real causal agent of this disease. It will be generally conceded that as regards diphtheria the theory that a microbe is the cause of the disease has passed from the realm of theory into that of observed fact. If one sees a murderer plunge a knife into the heart of his victim, it is a condition that confronts one and not a hypothesis.

Before discussing the present status of the germ theory in other fields it is worth while to ask ourselves why bacteria and other microbes should ever do such harm as they have been shown to do in diphtheria and some other diseases. They are plainly not consciously malicious creatures striving to kill human beings for their own pleasure or advantage. Biologists say that the driving forces of all living organisms are hunger and love. Bacteria have never been shown to possess any form of sex life; their primary need is that of nutrition. It is to satisfy this insatiable need for food, for available potential energy, that we find bacteria distributed so widely over the earth's surface, in deep polar seas, in tropical jungles, in the soil, in manure heaps, in milk, in distilled water, on fruits and vegetables and in the living bodies of plants and animals. When bacteria are living in the alimentary tract of the higher animals they may, like the species found in the normal intestine, not only cause no injury but even be of some benefit

to the host that gives them food and shelter. Other species, equally innocent in intention, may chance to produce substances that seriously injure the host-organisms. In satisfying their need for food it may happen that they secrete or excrete poisonous substances, or that they break down food substances into toxic compounds, or that their own cellular substance is poisonous for man in the way in which certain mushrooms are poisonous. It may be that in some instances the defensive efforts of the host to protect itself against the invading parasites cause the latter to strike back, as it were, by generating substances that injure the attacking cells of the host. In the majority of the best studied diseases, however, the toxic substances formed by microbes appear to be mere incidental products of the attempt to satisfy hunger, and are not weapons of warfare. The known bacterial toxins are seemingly of no benefit to the microbe producing them. Quite the contrary! It is a disadvantage to the diphtheria bacillus to kill the host that is giving it abundant nourishment under ideal conditions of temperature and moisture.

In but few diseases has the part played by an invading microbe been so completely worked out as in diphtheria. Each infection is a problem by itself and the unsolved questions in respect to many maladies are likely to occupy investigators for years to come. The evidence that a specific microbe is the cause of a specific disease varies in kind and in cogency. Let us consider briefly a few instances.

Tetanus.—The drumstick-shaped bacterium believed to cause tetanus or lockjaw is almost as well known and studied as the diphtheria bacillus. The so-called

tetanus bacillus is not a whole-hearted parasite. Its natural habitat appears to be the intestinal tract of some of the higher animals such as the horse, where it leads a harmless existence. Only rarely when it happens to be pressed deep into a wound—for it multiplies best in the practical absence of free oxygen and is not able to grow on the surface of the body—does the tetanus bacillus grow in the tissues. Unfortunately, when it does grow so it generates a toxin which has a combining affinity with the cells of the nervous system and by its action produces the serious nervous symptoms of lockjaw. The evidence that this drumstick-shaped bacillus is the cause of tetanus is just about as convincing as the evidence that the Klebs-Löffler bacillus causes diphtheria. The character of the wound which gives rise to tetanus is significant, not a clean razor cut or saber thrust, but a wound made with rough jagged objects—such as the traditional rusty nail or the fragments of toy pistol caps—which force foreign matter deep into the wound away from the air. This corresponds with what we know of the relative abhorrence of the drumstick-shaped bacillus for free oxygen. Moreover, examination of the wound in patients with lockjaw shows the presence and apparent multiplication of this characteristic microbe. As with diphtheria the so-called tetanus bacillus has been grown in pure cultures in test tubes, has given rise to the disease when introduced on a splinter of wood into mice, has been shown to generate a powerful soluble toxin which is able by itself to reproduce the symptoms of the disease. As with diphtheria the injection of tetanus toxin first in minute, then in gradually increasing, doses into a horse leads to

the formation of an antitoxin which has been used in the prevention and cure of tetanus. Although, owing to certain technical difficulties, tetanus antitoxin has not been so marvellously successful in the treatment of the disease as diphtheria antitoxin has been in diphtheria, it has nevertheless proved of great practical value. All things considered, the reasons for believing the drumstick-shaped oxygen-shunning bacillus to be the cause of tetanus are so impressive as to command general acceptance.

Anthrax.—In a few other infectious diseases the evidence that a specific germ or microbe is the cause is so varied, so extensive, indeed so overwhelming that no competent person can retain a reasonable doubt as to causal relationship. There are still other diseases in which investigators generally believe that the responsible microbe has surely been discovered, but in which our knowledge of the whole chain of events is not so complete as in tetanus and diphtheria. A classical example of the latter group is anthrax or splenic fever. This disease, famous as the first infection definitely traced to a microbe, primarily affects cattle and sheep and is sometimes transmitted from them to man. A rod-shaped germ, usually found in great abundance in the blood and spleen of animals suffering from anthrax, was cultivated outside the animal body by Koch, and shown by him to be in all probability the cause of the disease. His work published in 1876—just 50 years ago—marks a red-letter day in the history of the germ theory. It is not too much to say that the establishment of the germ theory as one of the great generalizations of science dates from that time. It is now almost axiom-

atic that all infectious diseases are due to some sort of germ or microbe even though in a given instance the individual microbe may not yet have been identified. In the case of anthrax there are still important gaps in our knowledge. We do not today know how the anthrax bacillus produces injury and death. Thus far no one has succeeded in demonstrating the presence of a soluble poisonous substance in cultures of this organism, and the mechanism of the disease process remains quite a mystery. One hypothesis is that the anthrax bacillus by its rapid and enormous multiplication clogs the small blood vessels and interferes mechanically with the circulation. There are, however, serious objections to this view. We have to admit that we do not yet understand some of the most important features of anthrax infection.

Other Infectious Diseases; Typhoid.—Among the diseases of man in which some particular microbe with definite and easily recognizable characters is believed to be the causal agent are tuberculosis, typhoid fever, syphilis, gonorrhea, epidemic meningitis, pneumonia, Asiatic cholera, bubonic plague and a number of other widespread and fatal maladies. No modern investigator doubts that the germs associated with these infections by bacteriologists are the real causal agents. At the same time there are loose ends in almost every instance.

Typhoid fever, for example, and others among those named above are not diseases from which the lower animals naturally suffer; so animal experimentation is largely blocked or forced into roundabout and not wholly satisfactory methods. Enormous numbers of typhoid bacilli may be fed to cattle, for example, as

well as to the ordinary laboratory animals such as rabbits and guinea pigs, without producing any ill effects. The germs appear to die out in the intestinal tract of those animals, whereas in man they spread through the body and multiply in the blood and tissues. Even in typhoid, however, the evidence is very strong that the so-called typhoid bacillus is the cause of the disease. The germ can be readily grown in beef broth and is cultivated in every bacteriological laboratory. With these pure cultures and in localities where no case of typhoid has existed over a long period, accidents to laboratory workers, such as sucking into the mouth a few drops of broth culture, have occurred, and more or less successful attempts at suicide have been made. The voluntary or involuntary swallowing of a very small quantity of these bacilli—whose ancestors originally came from the blood or spleen of a typhoid patient—has been followed in a score or more of cases by a typical attack of typhoid. While lacking the force of a carefully planned and controlled experiment, these laboratory accidents are highly significant. The case for believing this specific bacillus to be the cause of typhoid does not rest here. As well known, killed cultures of this same organism have been widely used for inoculation against typhoid. During the Great War typhoid was almost non-existent among the soldiers of the U. S. Army who had received the inoculation treatment, whereas in all previous wars typhoid had raged in every encampment. In the Spanish-American War in 1898, before anti-typhoid inoculation had come into use, there were within 3.5 months 20,738 typhoid cases among 108,000 men; in the Great War during the entire war period there were only 1,529 cases among 4,128,478 men.

Here again the germ theory is justified by the results of its practical application. Typhoid fever has been prevented under conditions of great exposure, by practices undertaken on the belief that a particular germ is the exciting cause.

Malaria.—One remarkable outcome of the germ theory has been the discovery that a great variety of microscopic parasites, some of them with complicated life histories, are concerned in the production of diseases. The history of the malarial plasmodium, which passes its sexual phase in the body of the mosquito, its asexual in the blood of man, is generally familiar. We know that only certain kinds of mosquitoes can serve as hosts for the human malarial parasite—although others can furnish food and lodging for the parasite of bird malaria; we know that the extraordinary periodicity of the malarial chill coincides with the regularly recurring cycle of asexual development of the parasites in the red blood cells; we know that quinine cures malaria because directly or indirectly it kills the germ. While the human race is still far from being able to subjugate malaria completely, the prevalence of the disease in certain regions has been greatly reduced by measures taken in the belief that a minute parasite in the blood is the cause, that this parasite is transmitted by the bite of a particular kind of mosquito and that diminution of the number of mosquitoes by draining, oiling, or sprinkling the breeding places with arsenic will lessen the chance of infection.

Plagues.—In the whole field of insect-borne disease the practical triumph of the germ theory has been highly spectacular. Yellow fever has been almost entirely banished from the American continents by the simple ex-

pedient of instituting anti-mosquito campaigns in certain districts. Typhus fever, the once terrible pestilence of crowded jails, ships, army camps and city slums, can be held in check by keeping the human body free from lice. Bubonic plague, the "Black Death" which scourged Europe in the Middle Ages and still kills its hundreds of thousands yearly in British India, can be prevented from sweeping over the world by the trapping, poisoning and killing out of the common rat, thus automatically reducing the number of rat fleas, the go-between in transmitting the disease from rat to man. The destructive and once highly dreaded disease of cattle known as Texas Fever is now no longer a menace to the cattle industry in the United States, since it can be readily prevented by "dipping" the cattle in poisonous washes and by other methods of eradicating the ticks which carry the parasite of the infection. The basic assumption of the germ theory that a self-reproducing micro-organism is the cause of each infectious disease has been brilliantly justified.

Unsolved Problems.—Unfortunately our knowledge in regard to a number of diseases is not so complete as it is with respect to diphtheria and tetanus, or even to anthrax, tuberculosis and malaria. There are several diseases in which a specific microbe has been found, but not identified to the satisfaction of all investigators as the cause of the disease; such are influenza, measles, acute poliomyelitis and epidemic encephalitis. In two diseases long amenable to preventive methods, smallpox and rabies, no micro-organism has yet been discovered which in the opinion of any considerable number of investigators meets the requirements of a causative agent. A

large group of "filtrable viruses" exists in which no definitely shaped microbe can be made out, only minute dots being visible in the field of the most powerful microscope. Foot-and-mouth disease of cattle, which is sometimes transmitted to man, is an example of an infection of this class. The "mosaic disease" of the tobacco plant is apparently due to a filtrable virus. Some investigators question whether certain alleged viruses are really living organisms and not rather chemical substances similar in their nature to enzymes. In some instances the "germ theory" plainly has only the status of a theory and can claim little or no basis in fact. Cancer is believed by a few to be a germ disease, but this opinion is not shared by the great majority of investigators. There is much uncertainty as to whether all varieties of the common cold have a microbic origin and are genuine specific infections.

There is no doubt that the germ theory of disease has been the most fruitful generalization in the history of medicine in so far as heuristic influence is concerned. It has also led to practical methods for controlling disease which have already done much to lengthen human life and decrease human suffering. As it stands at present the theory that infectious disease is due to microbes has been proved in some instances, is almost certainly true in others, is a valid working hypothesis in still others, and is on trial in a few cases where the question of infection itself is doubtful. Pasteur's interest in the phenomenon of fermentation, apparently first roused by his study of the chemistry of racemic acid, has had unexpected reverberations, undreamed of even by that man of great vision.

Chapter IX—Article 2

IMMUNITY—THE CHEMICAL WARFARE OF EXISTENCE

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All living things are forced to go through life beset by enemies that are constantly seeking to destroy them. Sometimes they defend themselves by physical methods, and are equipped to do this by means of weapons, such as horns, teeth and claws, or by such defensive armor as shells, hides and furs. But to a much larger extent the defense is chemical, although usually the chemical defenses are not so obvious as the physical defenses. There are exceptions to this statement, however, and chemical warfare is neither a human nor a recent achievement. Our chemical warfare service was long antedated by its prototype, the skunk. The smoke screen of the navy and the aeroplane is but a recent imitation of the ink clouds of the squid. Even the primitive chemical

warfare with the poisoned arrow of the aborigines was long antedated by the venomous snakes, whose beautifully functioning apparatus for hypodermic injections is a marvel of efficiency and clever construction.

But our most dangerous and persistent enemies are not those that can be seen with the naked eye. Even during the holocaust of the World War we had to pause to recognize the superior dead-lines of the invisible virus of epidemic influenza. Pneumonia still retains Osler's title of "Captain of the men of death." Streptococci beset us continually, and piece by piece destroy our vital organs until we succumb to heart and kidney diseases. The number of these invisible enemies is legion, and against them invisible agents must be used for warfare. We are eagerly seeking and hoping to find new artificial means for chemical warfare against these invisible parasitic enemies, such as already have been brilliantly found for a few, including quinine for the malarial parasite, arsphenamine (salvarsan) for the spiral germ of syphilis, and a few other drugs.

Natural Chemical Defense.—But we must appreciate that nature has already provided us with remarkable means of defense against the swarming hosts of bacteria and protozoa that assail us constantly, as attested by the fact that somehow most of us do survive their attacks for some years or decades, even though eventually they do overwhelm us. We all are attacked by infections at some time in our lives, whether it be measles, scarlet fever and the rest of childhood's enemies, or the common pus infections, colds and tuberculosis, from which none completely escape. These diseases have the power to kill, as we sometimes sadly learn, but generally the afflicted victim wins the battle by means of unseen

agents, by means of chemical warfare, and comes through to meet other invaders that enter his body with every breath he draws, with every mouthful of food or drink. Throughout life, on his skin and on the mucous membranes of his mouth and intestines, lie myriads of unseen enemies, potentially capable of overcoming him, but rarely succeeding, largely because of his chemical defenses.

Perhaps the simplest example of chemical defense that can be cited is the gastric juice. In the stomach, through which must pass to the absorptive surfaces of the intestines the myriads of bacteria that our food too often contains, is secreted so crude an antiseptic as hydrochloric acid, in concentrations great enough either to kill or injure most of the bacteria which are capable of attacking the animal body. The weakened survivors of this acid bath, passing on into the intestines, are there met by other chemical defenses, such as the bile, which dissolves or attenuates some sorts of bacteria, and the products of the natural bacterial flora of the intestines, which are poisonous to most of the unnatural occupants that enter this chemical vat or septic tank which stretches through our deepest recesses.

When a cross-channel swimmer is anointed with a heavy coat of grease for protection from the salt water, he, or often she, is receiving merely an exaggerated dose of what the skin normally does for our protection. In the skin are glands which elaborate an oily secretion, composed largely of esters of cholesterol, which partially waterproof the skin and which greatly help to make the body impervious to bacteria. The mucus-secreting glands of the nose and throat also help in defense by providing

a protective coat of mucus to entangle bacteria and to keep them away from the body surfaces.

Defense by White Blood Cells.—But often enough, despite these chemical defenses, and many physical defenses that I have not described, harmful bacteria do get across the boundaries, and then the warfare against the invader begins in earnest. In large measure this is a chemical warfare, just as the attack by the invading germ is a chemical attack, for few invading parasites do harm by physical means to any recognizable extent. If we study the field of battle with the microscope we may see what looks very like a true warfare, with organized hosts drawn up to repel the invading enemy. These defending forces consist of body cells, especially the white cells or leucocytes of the blood, and the free wandering cells of the tissues, which accumulate at the point of danger and usually prevent the spread of the invaders. Such contests are probably going on all the time here and there along the vast extent of our body surfaces, usually so successfully that we are not conscious of them. Only when an unusually heavy attack has to be overcome do we recognize the occurrence of the process as an area of inflammation. Now, although this means of defense by wandering cells may look like a physical defense, on examination we find it to be largely chemical. The white cells which migrate from the blood out into the inflamed area are drawn from remote parts of the body, especially from the marrow of the bones. As there is no physical connection between these wandering cells and the inflamed area, by nerves or other such means, the communication must be by chemical means through the fluids of the body.

The apparently intelligent migration of the cells to

the point of infection also seems to be determined by chemical agencies. It would seem that the products of the infecting bacteria act on the surface of the cells to lower the surface tension, that is, the force which draws the surface into the smallest possible area which is the sphere. When the surface tension is lowered on one side, the tension of the other surfaces forces the contents of the drop or cell into this area of lowered tension, bulges out this part of the surface, and moves the cells in the direction of the bacteria. This process may continue until the bacteria are actually surrounded by the flowing cell, and within this cell the bacteria may be digested and destroyed. We believe that wandering cells behave in this way on account of chemical changes which alter the surface tension, because we can so beautifully imitate their behavior with artificial cells, as was shown by Rhumbler with his artificial amebae.

Artificial amebae, made by suspending a drop of oil in dilute glycerin and alcohol, behave startlingly like living amebae or leucocytes in response to chemical stimulation. For example, if a little alcohol is brought in contact with one side of the oil drop the surface tension will be lowered at this point, a bulge will be seen, and the artificial ameba will move forward into this bulging part, much as the real ameba crawls forward into the arms that protrude from its surface. Even such specialized processes as the taking and rejecting of food may be imitated by these artificial cells; thus, a drop of chloroform will take in and dissolve a piece of shellac or wax, but will reject and cast out a bit of glass or wood. Or, the building of shells by forming a layer of grains of sand over the surface, which is character-

istic of some species of amebae, can be closely imitated by a drop of oil suspended in water and alcohol.

Inflammation.—From such experiments as these, and from many observations on living cells, it seems probable that the striking defensive processes carried on by the cells in inflammation are essentially chemical processes, which result in chemical destruction of the invading bacteria within protective cells. Indeed, typical inflammatory reactions may be produced by the presence in the tissues of relatively simple chemical substances, such as turpentine, iodoform, and cantharidin. Just what chemical properties a substance must have in order to incite an inflammatory reaction, with accumulation of wandering cells, is not yet known, and this is an important problem that awaits solution. It is important because initiation of graded inflammatory reactions is sometimes desirable; because control of inflammation is often necessary; and because we may wish to avoid producing inflammation with substances that have to be injected into the body. About all we have learned so far is that fat-solubility seems to be an essential feature of inflammatory agents, which is perhaps related to the fact that the properties of the surface membranes of the cells seem to depend largely on their fatty content.

Immunity Conferring Chemicals.—The defense offered by the wandering cells of the body is by no means all that the body has at its command, and so it is able to overcome bacteria that escape these cells and also to neutralize the soluble poisons of the bacteria which are their chemical weapons of attack. Soon after Metchnikoff had shown the power of the cells to take up and destroy bacteria, Nuttall and Buchner showed that the

fluids of the blood, even when deprived of all the cellular elements, contain substances that have marked power to destroy certain bacteria. When it was further shown that the blood of animals made immune to any species of bacteria commonly has an increased power to destroy this same sort of bacteria, and when Behring showed that the serum of animals immunized to the poisons of diphtheria and tetanus can neutralize much more of these poisons than is neutralized by the serum of normal animals, the modern science of immunology was born. Since those thrilling days of the early nineties, when the aforementioned scientists and their colleagues were eagerly disputing whether immunity and resistance depend upon the action of the cells or upon the humors of the body, and turning up new facts that eventually proved them both to be right, we have added not a few more new facts and plenty of theories, without as yet having very clearly explained anything. But at least it can be safely said that *the processes of immunity are essentially chemical processes,* especially in the broad sense which recognizes physical chemistry and colloidal chemistry as but different branches of a common discipline. We do not as yet know exactly how the various reactions of immunity are accomplished, nor the chemical nature of the constituents of the blood that are responsible for the observed processes, but we are sure that the reagents concerned are substances endowed with active chemical properties and produced through chemical activities of the tissues of the body. We have not as yet been able to isolate them in pure condition to determine their chemical composition or structure, but can only study their properties and recognize their existence by observing the reactions or effects that they pro-

duce. Therefore the chemistry of immunity is on quite the same plane as the chemistry of the enzymes, such as pepsin, which are recognized by what they do, in the face of our complete ignorance as to just what they are.

IMMUNOLOGICAL DEFENSE

The things that are done by the blood serum of immune animals, in greater degree than by the serum of normal animals, and which we call the immunological reactions, are briefly described in the following paragraphs.

Neutralization of Toxins by Antitoxins.—A few bacteria, of which the most important are those responsible for diphtheria and tetanus, secrete soluble poisons that cause the chief manifestations of the disease for which each is responsible. The blood of man or animals recovered from infection with these bacteria has an increased power to neutralize this poison, and a still much greater neutralizing power is obtained by artificially immunizing with the toxins. The venoms of poisonous snakes and insects, and a few plant poisons, such as the poison of castor oil beans (ricin), also exhibit this same property of developing a high antitoxic power of the blood. Unfortunately, the germs causing most infectious diseases do not owe their toxic effect to poisons or toxins against which antitoxins can be produced, or we could control them as well as we now can control diphtheria and tetanus, concerning which it is not too much to say that all deaths are avoidable and unnecessary.

The chemical defense of the body, such as it is, against the other more numerous class of parasites, is waged by other means than the formation of antitoxins. We recognize two other chief defensive mechanisms.

Destruction of Bacteria by Serum.—Since the days of John Hunter (1728-1793) it has been known that blood has more power to resist decomposition than other fluids of equal nutritive value, but it was not until 1886 that Nuttall and others showed that whole blood and blood serum have actually a definite although limited power to destroy bacteria. Soon it was found that the blood of animals immune to a given bacterium might show a greatly increased power to kill this particular sort of bacteria (*bactericidal* power). In the case of certain bacteria, notably the one causing cholera, the immune blood actually dissolves the organisms (Pfeiffer). This process is called *bacteriolysis.* Pfeiffer and others then showed that this destruction was accomplished by two components of the blood serum, one of which united with the bacteria and produces some change whereby they can be killed or dissolved by the second bactericidal or bacteriolytic agent. Just what these agents are we have failed to learn in forty years of study, but for purposes of discussion we have endowed them with names. We call the sensitizing agent the *immune body,* and the destructive, enzyme-like agent which completes the bactericidal action, the *complement.*

We have now mentioned two ways in which infecting germs are attacked or held in check, but not all bacteria seem to be susceptible to these two methods. Some bacteria seem to be so resistant that they withstand the bactericidal power of the blood despite the maximum amount of immunization. In this class come the bacteria responsible for the largest group of human ills, namely, the minute roundish *"cocci,"* which cause boils, abscesses, tonsillitis, peritonitis, erysipelas, pneumonia, meningitis, and a very large share of all known infec-

tious disorders. If the cocci could be eliminated from human affairs we should really have remarkably little left in the way of disease, for besides the diseases mentioned above, they are probably indirectly responsible for most of the chronic diseases of hearts and kidneys, for rheumatic conditions, for many of the effects of tuberculosis, cancer and other diseases that the cocci do not themselves cause, as well as the many troubles that they incite unaided. To combat these troublesome parasites the body has to combine methods, and uses in conjunction the blood serum and the wandering cells of the body, as described below.

Destruction of Bacteria by Cells.—This is accomplished particularly by the white cells of the blood, the leucocytes, and by the free wandering cells of the tissues, but perhaps other cells may take on this same function to some degree. As was said in discussing inflammation, chemical changes at the site of infection cause a flowing of these cells to the point where the infection is located, this process being called *chemotaxis*. When the wandering cells reach the bacteria they often take them into the cell body (this process being called *phagocytosis*) where the bacteria may be killed and dissolved. This action was discovered by Metchnikoff, the great Russian scientist who succeeded Pasteur as director of the Pasteur Institute in Paris, and for some years there was much discussion as to whether immunity was accomplished by the cells or by the serum. Eventually, Wright of London pointed out that the phagocytic cells could not take up bacteria unless these had first been acted upon by serum, and that the serum of immune animals has a much greater power to prepare or sensitize bacteria for phagocytosis than normal blood.

Thus it was shown that the destruction of this resistant type of bacteria requires the combined action of the cells and of some agent present in the blood serum, which, since it prepares the bacteria for phagocytosis, Wright christened the *opsonin* (meaning *I prepare food*).

While the three processes described above include the chief immunological phenomena observed in the defense of the body, they by no means include all the known reactions of immunity, for these reactions are not limited to the defense of the body against invading germs, but are fundamental biological phenomena of wide application. Similar reactions, indeed, may be manifested against any and all sorts of foreign proteins that have been made to penetrate the epithelial surfaces that normally protect the body. Of course, in nature the foreign proteins most likely to invade the body are the bacterial proteins and the proteins of the simpler animal parasites, such as those of malaria and other protozoan diseases. But when in the practice of medicine there was introduced the hypodermic syringe, and innumerable substances were injected into the blood and tissues, it was found that important reactions often resulted, sometimes with highly undesirable results. Also it was found that immunological reactions besides the three mentioned above could often be elicited in one way or another, and some of these reactions have been found to be of great value not only in medical problems, but in problems of bacteriology and biochemistry. Some of these other reactions we describe briefly below.

Agglutination.—When the blood serum of a man or animal is added to a suspension of bacteria, the bac-

terial cells may clump into groups which tend to settle to the bottom of the fluid. If the bacteria are motile they lose their motility. If the blood is from an individual immunized to this species of bacteria, it will exhibit a much heightened capacity to cause this agglutination. This is the principle on which is based the well known Widal test for typhoid fever, the blood of people with typhoid showing an increased power to agglutinate typhoid bacilli. Similar agglutination reactions can be obtained against any sort of cells, such as red blood corpuscles, by immunizing an animal with either these cells or their protein constituents.

Precipitation.—If instead of whole cells, solutions of proteins are tested, the protein particles will aggregate and a precipitate form. This reaction is evidently the same as that of agglutination, except that it is applied to the dissolved proteins instead of to formed cellular aggregations of proteins. It has also been found to be of great value in identifying proteins in minute quantities both for medico-legal purposes and in biochemical studies.

Anaphylaxis.—If the foreign protein is injected into the body of an animal which has been sensitized by previous injection of a minute amount of the same protein, the animal may exhibit a profound reaction, often fatal. Unbelievably minute amounts of protein may accomplish this sensitization (*e.g.*, of egg albumin, 0.000,000,05 gram) and hence it serves as a remarkably delicate test for the presence or character of proteins in a solution.

Several other reactions have also been observed, such as the *complement-fixation* reaction which applies the principle described as responsible for bacteriolysis by

serum, and on which is based the well known Wassermann reaction used in detecting syphilitic infection. It will not serve our purpose to go into further details concerning these, although they all demonstrate our main thesis, that the immunological reactions are chemical reactions, and thereby that *immunological defense is a chemical defense against a chemical attack.*

The immunological reactions, as chemical reactions, are of great value in the study of chemical problems when these are concerned with proteins. Because such infinitesimal quantities of proteins give these tests, the methods of the immunologists are far more delicate than the methods of the biochemist. For example, the biuret reaction will detect proteins in a dilution of one in one thousand or so, the precipitin reaction will detect one part in ten thousand or even in one hundred thousand; the complement-fixation reaction detects one part in a million, while by the anaphylaxis reaction less than one millionth of a gram of protein may be detected. But the superiority of the immunological reaction over ordinary chemical tests is much greater than is suggested by these figures, for the chemical tests merely tell that some sort of protein is present, while the serum reactions tell us just what one of all the infinitely great number of possible proteins is present in our solutions. This fact depends upon the uncanny specificity of the reactions. Not only can we tell, in medico-legal work, that a given blood stain was made by human blood rather than by the blood of a dog or a cow, but we can get evidence on biological relationships. For example, we can show by these reactions that the blood of the chimpanzee and orang-utan is more closely

related to human blood than to the blood of the common monkeys. These reactions will also pick out a single protein present in minute amounts in a mass of other proteins. For example, in the lymph coming from the thyroid gland can be identified the minute quantities of thyroid protein present therein. Some of the manifestations of this immunological specificity are so remarkable as to stretch our credulity, were they not attested by competent scientists who control their observations carefully. Especially are such reactions seen in man himself in his manifestations of hypersensitivity or anaphylaxis, most familiar in the form of hay fever, asthma and hives. These are commonly reactions to foreign proteins, such as pollens and food proteins, but sometimes proteins from other sources, and each of these conditions is the result of reactions to special proteins. The person sensitive to ragweed pollen may not react to rye pollen, the victim of guinea pig asthma may ride horseback with impunity while the subject of horse asthma may play with guinea pigs. We even read of a woman reacting to her husband's dandruff while not affected by the dandruff of her neighbor's husband, or the person sensitive to hen's eggs who could eat the meat of roosters but not the meat of hens!

Biological Importance of Protein Specificity.—The remarkable specificity of these reactions seems to depend on the corresponding specificity of the proteins. In fact, it is probably justifiable to look upon immunology as merely one field of protein chemistry. The immunological reactions seem to be incited by the presence within the fluids or tissues of living animals, and possibly even of plants, of proteins foreign to these fluids or

tissues. We are not sure that anything but proteins can cause any such reactions. Even the tolerance to such habit-forming drugs as morphine and alcohol, which looks something like immunity, does not depend on any demonstrable change in the blood serum like that observed in the immunity to snake venom or typhoid bacilli. And indeed of all biological substances, only the proteins are of sufficient variety to explain specificity of such infinite variety as has been mentioned above. Proteins being complexes of amino acids, most of the 20 or so which have been found in proteins being present in every protein, the possible number of distinct and different proteins that might exist in nature is enormous, and entirely sufficient to explain what we know of the range of immunological specificity. Abderhalden has calculated that 20 amino acids could form at least 2,432,902,008,176,640,000 different compounds, and this without including the enormously greater number that might be made by varying the proportion of the different amino acids in a single protein. Certainly this fact of itself is strong support for the hypothesis that immunological specificity, and most of the other features of biological specificity, such as heredity, must depend on the proteins. They alone could furnish the infinite variety of modifications found in the multitudes of different living creatures and in their life processes. And, moreover, we find that to guard the specificity of all living forms, elaborate provisions have been taken to see that foreign proteins do not enter the cells or the circulating fluids until they have been robbed of their foreign character, through digestive splitting into simple amino acid complexes that lack specificity.

The Chemical Nature of the Activating Substances Still Unknown.—Although we know reasonably surely that the substances which incite the immunological reactions are proteins, we have but little idea about the nature of the reacting agents in the serum. Many attempts have been made to isolate them, but without success. A pure antibody is as yet as unattained as a pure enzyme; indeed antibodies and enzymes resemble one another in many respects, especially in that they are so intimately associated with the proteins, although we do not know whether they actually are proteins with a special molecular structure or with some active radical attached. We do not even know whether each of the several reactions described depends on a special sort of antibody (*i.e.* antitoxins, agglutinins, precipitins) or whether there is only a single type of antibody which is demonstrable by these several different reactions. As far as chemical properties are concerned, we merely know that they are found in a certain part of the proteins of the serum, but we do not know whether the immune bodies are serum proteins modified by the process of immunization, or specific proteins formed in and secreted by the cells to unite with the antigen, or specific chemical radicals either attached to or forming part of the protein molecule. There is a striking similarity to be noted between antibodies, toxins, enzymes and insulin. Each of them acts in quantities that are so infinitesimally small that it is not possible to say whether the proteins which may usually be found in the solutions are themselves the active agent or merely contaminations. Especially similar are the antibodies and insulin, since each seems not to be the active agent

itself, but merely one that accelerates or makes possible the action of other agents. Thus, the antibody serves to activate the complement; the insulin serves to make possible the burning of sugar by the tissues. Neither of itself accomplishes the final reactions. The amount of material necessary to accomplish this in the case of insulin is similar to the extremely minute figures obtained in quantitative studies of toxins, etc.

Great Gaps in Our Knowledge.—The foregoing synopsis of our knowledge of immunity should serve to indicate that, despite the abundance of new facts that have been unearthed in forty years of study, we still are entirely ignorant concerning the most important of all the problems, namely, just what are the substances that enable us to resist and overcome disease, and how do they act? These problems are of vast practical importance for us. Up to the present time we have been enabled, through our study of immunity, to accomplish some tremendously important things, such as the curative treatment of diphtheria and the prevention of a few diseases, such as typhoid, smallpox, cholera and a few others. But this is only a beginning. Even with these diseases our results are as yet far from 100 per cent of perfection, and with the rest of the great category of infectious diseases we have made little or no progress. Pneumonia still leads in the causes of death. Peritonitis and other acute infections still kill myriads. An influenza epidemic coming next month would find us no better prepared to prevent or to cure an attack than did the last one, and so on. Our efforts to immunize accomplish at the best but partial success, and often they achieve nothing. Our few curative

serums, of which only diphtheria has a high proportion of success, are at most but feeble, dilute solutions of the protective agents, gleaned with difficulty from other animals and used in an unnatural environment. Our prospects of any great advance by means of improvement of the methods we now know and use seem but slight. We must have a new method and that must be furnished by the joint action of the chemist and the immunologist. When we learn just what these mysterious protective agents are, how they act, and how to produce them artificially at will, we shall not have to depend on the dilute solutions of these agents that we can extract from a horse or can cause to develop in a man's own blood, but we can give each patient as much as he needs of the active agent that has been prepared by synthetic chemistry. That seems to be the goal we must work towards, and the organic chemist who is studying the linkage of pyrrols, piperazines, and amino acids, unpractical as these tasks may seem, is probably quite as much a builder of the future immunology as the man who is investigating the serum reactions of the meningococcus. Emil Fischer and Kossel, through their studies of the composition of the proteins, are perhaps quite as directly the founders of the future immunology as Metchnikoff, Ehrlich and Bordet, who worked more directly with the living processes of infection and resistance.

How far we may be able to go through study of the chemistry of immunity we cannot now foretell. Possibly we may attain the conquest of all the infectious diseases by accomplishing artificially on a larger scale what nature achieves but partially. On the other hand,

we must recognize that perhaps the possibility of advance through immunological methods is limited. Certainly in nature it is highly limited, and, deplorably enough, it is particularly limited with some of the most common infections. We must keep in mind that the resistance the body develops to certain diseases is but transient, apparently often evanescent, especially with the diseases produced by the cocci. The victim of pneumonia may come down with a second attack before he is fairly through with the first, and after recovery from this disease he has not more resistance to pneumonia than other people, but apparently oftentimes he is more susceptible. The same is true of the victim of erysipelas, or tonsillitis, or of "boils," which everyone knows occur in "crops." Gonorrhea establishes no lasting immunity, nor does wound infection, appendicitis or peritonitis. Perhaps in these diseases we cannot hope for any great augmentation of immunity, when nature does so poorly despite abundant opportunities. Therefore any hypothetical future progress in the treatment and prevention of infections by artificial augmentation of natural defenses, should not divert us completely from that other line of progress, specific chemical remedies for infectious diseases. The possibilities for future accomplishments by drugs of this class are, as far as we can now see, unlimited. We have these two broad avenues of attack against these invaders of the sanctity of the human body. Let the size of the defending army of scientists and its equipment be augmented as rapidly as possible, for the invaders are well intrenched and destroying our fellow men and our live stock by the millions.

Chapter IX—Article 3

THE FIGHT AGAINST PUS ORGANISMS

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Dr. Churchman was associated with the Johns Hopkins Medical School as Instructor in Surgery, the Johns Hopkins Hospital as Resident Surgeon, and with Yale University as Professor of Surgery before he was called (1922) to Cornell University Medical College where he occupies the chair of Experimental Therapeutics. Besides other important contributions to medical knowledge, he is well known as the discoverer of the selective bactericidal properties of the dye, gentian violet. This and other dyes form the most promising starting-point in the quest for a remedy for general blood poisoning (septicemia) before which medicine stands almost helpless at present.

TYPES OF INFECTIONS WITH PUS ORGANISMS

The human body is constantly exposed to attack from a group of bacteria or germs which, because of their propensity to produce pus when lodged in the tissues, are often called pus organisms. These bacteria lie everywhere about us. They are present in the dust and on the surface of practically everything one touches. But they do little or no harm by merely lodging on an unbroken surface. It is only, as a rule, when the skin or mucous membrane is injured—as by a cut or pin prick—that they make their entry below the surface and begin their deadly work, although it does occasionally happen that they travel down a hair into the hair follicle and so start a "pimple" even in the normal skin.

Once the pus organisms have entered the cut or pin prick (the portal of entry as it is called) and have found

conditions favorable, they set up an infection. The body immediately reacts by organizing a defense; and a battle is then on between the invading organisms and the various protecting mechanisms which the body is able to muster. The subsequent course of the trouble will depend on which of the two gains the mastery. In many cases the barrier which nature erects is completely effective and the infection remains localized. Redness, swelling, and pain appear but, in the majority of cases, soon subside. Or an abscess may form; but, if it does, it "comes to a head," ruptures, the pus is discharged and healing occurs without any sign of the infection having spread far from the portal of entry.

In other cases Nature's defense is inadequate. The bacteria spread beyond the point of first invasion. They cause inflammation at a distance from the original injury. They may even enter the blood stream and be distributed throughout the body.

We may thus distinguish three main types of infection with pus organisms: (1) local infection, usually with abscess formation, (2) spreading infection, and (3) blood infection, blood poisoning or septicemia.

Now these three types of infection with pus organisms have to be fought in different ways. In the case of local infection it is often possible completely to excise the infected tissues much as one would lop off a rotten branch from a tree. The operation of appendicectomy is a procedure of this sort. The surgeon cuts through the abdomen, finds the appendix, removes it and thus relieves the body of the inflamed tissue. But in many cases this is either impossible or inadvisable. We could not, for example, cut off an arm because of an abscess, and in such cases we do what we can to aid nature by incising

the abscess, thus allowing the pus to drain out and the process of healing to proceed. Incision and drainage, if properly carried out and not too long postponed, are usually effective, and even spreading infection may, in the majority of cases, be successfully controlled by surgical means. If, however, the body defenses have broken down and bacteria have reached the circulation in large numbers, septicemia or blood poisoning occurs. In these cases widespread damage is done by the bacteria, infections are often set up at inaccessible points (like the interior of the brain) and surgery becomes more or less helpless.

In dealing with all these three types of pus infection —local infections, spreading infections, and general infections—medicine leans hard on chemistry. It is our present purpose to inquire into the status of this alliance and to indicate its alluring future.

The Selective Powers of Living Cells.—The whole machinery of vital activity is so marvelous and—despite all that we know of it—so far beyond our comprehension, that it is perhaps absurd to pick out any one set of phenomena as more remarkable than another. But if we may make a choice of this sort, the phenomena of vital selectivity are surely of a kind to fill with wonder the soul of any one at all capable of appreciating their significance. By reason of a chemical structure of unbelievable complexity and infinite exactness each cell of the billions of cells of the human body is able—by a process which corresponds closely to an act of will—to select from the thousands of chemical substances offered to it only certain ones and to reject all others. We inject cocaine, for example, into the skin. By reason of a sharp chemical affinity which this drug has for

sensory nerves, these nerves become paralyzed, while the motor nerves—although they appear to be similar in structure—are unaffected. If it is the finger into which the drug has been injected this member feels wooden, numb, dead. Painful sensations like the prick of a pin, or a cut, will not be appreciated, but the motions of the finger will in no way be interfered with, since the drug will have had no effect on the motor nerves. Indeed, the cocaine will not even have affected all the sensory fibers, for sensation of heat and cold will be preserved. Only pain and touch sensations will be abolished. It is very much as if in a cable made up of yellow, black, red, and blue strands some chemical were known capable of having a striking effect on the yellow and black strands but incapable of affecting the red and blue.

The same phenomenon of chemical selectivity is beautifully illustrated by the case of curare. This is the famous poison with which the aboriginal tribes of South America tip their arrows. Curare acts in a very peculiar way on the body musculature. Two types of muscle are present in the human body, and these may be readily distinguished from each other by their characteristic appearances under the microscope. One type is known as "striped muscle." This type is found in the heart and in the muscles, like those of the limbs, which are under the control of the will. The other type is known as "smooth muscle," over which the will has little or no control. This type is found in the intestines, the blood vessels, etc. Now curare paralyzes very readily the terminations of nerves in all striped muscular tissue (except that of the heart!): and it therefore causes a general paralysis of all voluntary movements, so that the poor victim of arrow-poisoning lies helpless, because

motionless, on the ground. But (unless the dose be very large) the innervation of the smooth muscles is unaffected. The strict demarcation of the action of curare is seen very distinctly in organs which consist partly of smooth and partly of striped muscle. Thus, in the esophagus the striped muscle fibres of an animal which has received curare no longer respond to stimulation while the smooth muscles continue to respond as usual.

Perhaps the most striking of all the phenomena of selectivity furnished by chemicals acting in the human body is the paralysis of the forearm, producing the well known wrist drop (inability to raise the wrist) which is sometimes seen in those who (like painters) are exposed over long periods of time to the absorption of lead. In this form of lead poisoning the absorbed metal paralyses all the numerous muscles on the back of the forearm (i.e., those which raise the hand on the wrist) with one single exception. This muscle—although identical in structure and similar in function to all the others —is always unaffected. Some fine chemical difference in the makeup of this muscle or in the nerve fibrils which motivate it—a difference we are quite unable to detect and can only hypothecate—makes it impossible for lead to combine with the tissues and cause paralysis.

Chemical Selectivity and Drugs.—We have dwelt at some length on the phenomenon of selectivity because it underlies the whole of drug therapy and more particularly because—since the demonstration by Ehrlich and others that selectivity could be modified somewhat at will by chemical manipulation of the molecule—it underlies the whole modern attack against pus organisms by means of synthetic antiseptics. The intelligent use of drugs depends on the wise choice of substances capable

of affecting the particular organ we wish to reach, and in the way we wish to affect it, without affecting, or at least without damaging, other organs.

For there is another form of selectivity which is as striking as any we have mentioned and which directly concerns our present inquiry as to the treatment of pus infection by chemical means. This is the selective affinity which certain drugs exhibit in their behavior toward body cells on the one hand and disease producing micro-organisms on the other. This type of selective activity can be well illustrated by the case of quinine. This drug, like most substances which kill micro-organisms, is a general protoplasm poison and is capable also of killing the cells of the body. It is, however, selective in its action since it is far more powerful against the plasmodium of malaria than it is against the tissues of the human body. If, therefore, we administer quinine to a malarial patient in proper dosage we can kill every single invading micro-organism without doing any appreciable harm to the patient; and the conquest of malaria by quinine is one of the most brilliant of all therapeutic successes.

Another equally brilliant success is presented by the case of arsphenamine (introduced as salvarsan). Here we have a substance whose selective activities have been actually created by chemical manipulation, to meet certain therapeutic demands. Starting with atoxyl, a substance which had been shown experimentally to have some therapeutic promise but which also had fundamental drawbacks, Ehrlich proceeded to diminish the defects and augment the virtues. This he was able to do by means of highly ingenious and exact experiments, in which the molecule was actually molded, through chemical substitutions, to meet the needs of the case.

By this success Ehrlich made a contribution of the first magnitude to the treatment of syphilis. But he also founded a new science, the science of chemotherapy—the underlying principle of which is that medicine need not simply accept drugs as they occur in nature, with all their defects, but that she can through chemical means, the general nature of which is already understood, modify the selective capacities of the substances somewhat at will. Modifications of this general nature form the basis of most of the investigations which are at present under way in this field, and which have for their object the improvement of the treatment of pus infections by synthetic means.

Disadvantages of Antiseptics Now in Use.—Now, pus infections differ fundamentally from the diseases, malaria and syphilis, for which the two drugs just mentioned (quinine and arsphenamine) are specifics. The organisms which cause syphilis and malaria are protozoa, that is to say, unicellular organisms low in the *animal* kingdom. Pus infections, on the other hand, are caused by bacteria, which are unicellular organisms low in the *vegetable* kingdom. Unfortunately the chemical substances which are so effective in combating diseases caused by protozoa have not proved of value in fighting infections caused by bacteria. This difference arises from the phenomenon of selectivity which we have been discussing. Quinine and arsphenamine are more toxic for protozoa than for the cells of the body; but in order to kill bacteria they would have to be administered in doses which the patient could not tolerate.

A number of antiseptics which can be used against pus infections of the local or spreading types are, however, known. Within limitations these substances are of

great value. When a surgeon, for instance, wishes to excise a localized infection (like an appendix) in the manner we have already discussed, he must, before he begins to operate, destroy all bacteria on the skin of the abdomen. Otherwise the pus organisms lying on the skin would be carried in by the knife and would start an abscess in the skin. For this reason surgeons generally paint the skin with iodine before operating. Iodine kills all the bacteria which it reaches and, while it does some damage to the skin, this is so slight as to be negligible. For purposes of skin disinfection iodine is therefore very satisfactory. But in many parts of the body it is extremely irritant. It cannot, for example, be used in the eye, for here, while it would still kill the bacteria, it would also cause damage to the tissues.

Similarly if we have opened an abscess we can hasten healing by washing out the cavity with an antiseptic. For this purpose bichloride of mercury is often used and it is a drug of great value. It is also, however, a powerful poison and cannot be used in very strong solutions; in some parts of the body it cannot be used at all.

For the treatment of local infections, therefore, and even for the treatment of spreading infections, chemistry has provided us with valuable substances which, within the limitations which have been pointed out, can be widely used. But neither iodine nor bichloride of mercury nor any other similar antiseptic can be used to combat generalized infections (blood poisoning). For if these substances were injected into the circulation (as arsphenamine can be injected in the treatment of syphilis or quinine in the treatment of malaria) serious damage and probably death would result.

The kind of difficulties with which one has to con-

tend in the treatment of infection by chemical means is well illustrated by the case of carbolic acid or phenol. When Lister first began to develop in surgery the ideas which had sprung from the epoch-making discoveries of Pasteur, carbolic acid was the substance with which he began to work. This is a most effective bactericidal agent. In strengths which do little or no immediate damage to the tissues it may be applied to the skin or to a wound surface, and it kills all the bacteria which it reaches. But it reaches by no means all the bacteria present. Not only has it little or no power of penetration, so that bacteria below the surface escape it, but it coagulates the albumin in the body fluids and thus actually erects a barrier against its own progress. Carbolic acid has an even more serious drawback. This drug when applied to the skin for a long time in weak dilutions is absorbed in small amounts and paralyses the minute blood vessels. The circulation is thus impaired and gangrene may result. The drug has a slight anesthetic action so that no pain is experienced and the patient is unaware of the damage that is being done. Before these facts were known it thus occasionally happened that gangrene occurred in a member which had been wounded and to which a dressing wet with weak carbolic acid had been applied. The use of this drug for such purposes of course had to be abandoned.

Synthetic Chemistry and Antiseptics.—Synthetic chemistry has come brilliantly to the aid of medicine by showing that just such defects in therapeutic substances as some of those which we have stated to be inherent in the more commonly used antiseptics, may be eliminated by molecular manipulation and that substances much more nearly ideal may in this way be pro-

duced. The case of procaine or novocaine may be cited as one of the early triumphs in the field of "made-to-order-medicines." Cocaine had proven of great value in the production of local anesthesia, but its use had been found to be attended by considerable risk. The structure of cocaine (see p. 464) was determined and then it was found possible to produce artificially a base analogous to cocaine but only about one-third as poisonous. This substance retained most of the valuable anesthetic properties of cocaine but was much less toxic for the human body. It is known as novocaine or procaine and has proved to be one of the most valuable of all drugs. By the use of this local anesthetic operations —even fairly serious ones—may be performed painlessly and absolutely without risk. The disagreeable effects of a general anesthetic are also avoided. For dental operations, such as the filling of sensitive teeth, or for tooth extractions it is indispensable. No one who has experienced the comfort of this scientific painless dentistry and can compare it with the butchery of previous days ought to need to be convinced of the value to human comfort of synthetic chemistry.

The successful work of Ehrlich in developing arsphenamine as a cure for syphilis by manipulation of chemical molecules, the successful development of novocaine by similar synthetic manipulation, and other trimphs of this kind stimulated investigators all over the world to undertake similar studies for the discovery of substances which would act toward pus organisms as arsphenamine acted toward syphilis; which could be used for local, for spreading, and for general infections; and which did not have the disadvantages already described as inherent in iodine, bichloride of

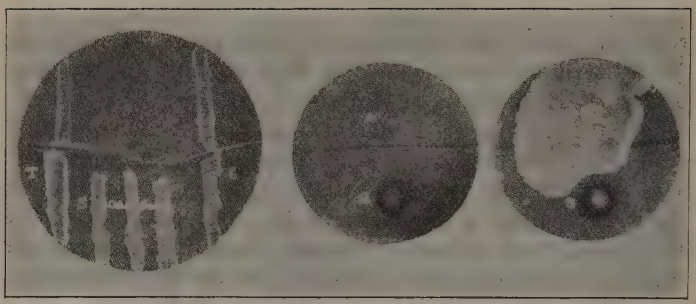
mercury, carbolic acid, and other commonly used antiseptics.

Synthetic Chlorine Compounds.—The antiseptic properties of chlorine had long been known, and in one form or another it had been widely used by physicians. Labarraque's solution—the form of chlorine devised by the great French scientist of that name—had had great vogue in France and elsewhere and this and other preparations of the element had been popular throughout the world in the fight against bacterial processes.

Chlorine is a powerful poison to all living matter, including bacteria, but it is intensely irritating and quite volatile. These characteristics greatly restricted the field in which substances setting free chlorine could be used and scientists therefore set to work to produce a compound in which the chlorine would be set free more slowly (i.e., which was less rapidly volatile) and which would be less irritating and so available for use on body surfaces. The now widely used Dakin's solution or Eusol, a neutral solution of sodium hypochlorite, is a substance of this sort deliberately concocted by chemical manoeuver to meet certain specific needs. It was widely used during the war in the treatment of infected wounds. Dakin also improved on this substance by producing organic derivatives of hypochlorous acid, for instance. Chloramine-T, $C_6H_5SO_2NH\,Cl$, a compound adapted to the same purposes as sodium hypochlorite but more stable and less irritating; and this substance is now quite generally used in the irrigation of wounds. By means of chemicals of this kind it is now possible to cleanse a dirty wound so that it may be at once closed up by suture instead of—as was always previously done—waiting for nature to heal the wound from the bot-

tom, a process that often takes weeks and even months. Many wounds were treated in this way during the war and endless amounts of time and suffering were saved. It is hard to imagine a prettier instance of the contribution of chemistry to human progress. Chloramine-T was a deliberate production of manipulative chemistry devised to meet a particular need. The war expanded this need to a colossal demand, and, thanks to the ingenuity and activity of chemists, medicine stood ready to handle the unprecedented situation effectively.

The Aniline Dyes.—Among the substances which were engaging the attention of investigators during the early days of chemotherapy were the aniline dyes. These coal tar products discovered by Perkin had been found to have, or at least some of them had been found to have, striking inhibitory powers over bacteria. And these powers were found to be highly selective. This proved to be strikingly the case with certain of the basic dyes of the triphenylmethane group. Gentian violet, a dye of this group, when incorporated even in small amounts in the media on which the bacteria were planted suppressed the growth of one large group of bacteria (the "Gram positive" group) but was without effect on another large group (the "Gram negatives"). This example of choice, another instance of the chemical selectivity we have already discussed, makes itself clearly seen in the kind of experiment illustrated on Plate I. A bacteriological plate has been made consisting of two portions containing different kinds of media. The lower portion contains plain agar (the medium used for the cultivation of bacteria) and the upper portion contains agar plus gentian violet in a dilution of 1 to 100,000. With a platinum needle aqueous suspensions of five dif-



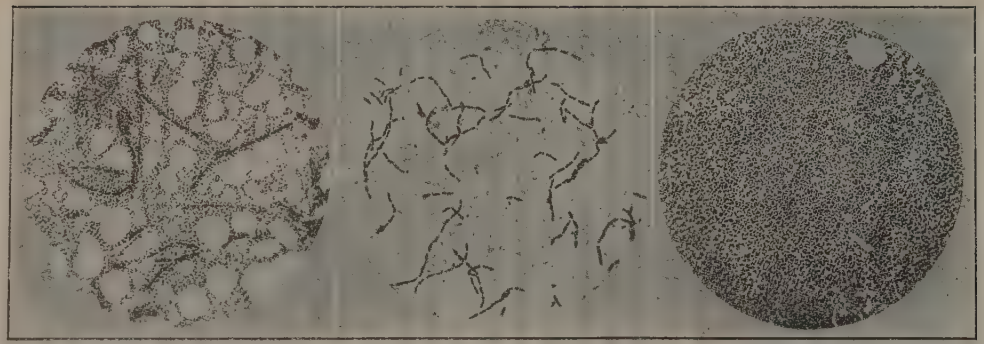
(*Courtesy of the Journal of Experimental Medicine*) (*Courtesy of the Journal of the American Medical Association*)

PLATE I

LEFT: THE PLATE HERE PHOTOGRAPHED CONSISTS OF TWO PARTS. THE UPPER PART (ABOVE THE IRREGULAR LINE WHICH CROSSES FROM SIDE TO SIDE OF THE PICTURE) CONTAINS AGAR-AGAR TO WHICH DYE HAD BEEN ADDED. THE LOWER HALF CONTAINS AGAR-AGAR WITHOUT DYE. FIVE DIFFERENT GERMS WERE PLANTED IN STROKES ACROSS THE PLATE, WHICH WAS THEN PUT IN THE INCUBATOR SO THAT THE GERMS COULD GROW. THE PHOTOGRAPH WAS MADE AT THE END OF 24 HOURS. THE TWO GERMS AT EITHER END HAVE GROWN AS WELL IN THE AGAR-AGAR WHICH CONTAINED DYE AS IN THE PLAIN AGAR-AGAR; WHEREAS THE THREE GERMS IN THE CENTER HAVE NOT GROWN AT ALL WHERE THE DYE IS PRESENT. THE PICTURES IN THE CENTER AND ON THE RIGHT SHOW THE RESTRAINING EFFECT OF THE DYE, GENTIAN VIOLET, ON THE GROWTH OF BACTERIA. THE PHOTOGRAPHS REPRESENT PLATES OF AGAR-AGAR, A GELATIN-LIKE SUBSTANCE ON WHICH BACTERIA GROW VERY WELL. TWO TINY DROPLETS OF A CULTURE OF BACTERIA, HARDLY VISIBLE, AT (A) AND (B), HAVE BEEN PLANTED ON THE SURFACE OF THE AGAR-AGAR; AROUND ONE OF THEM (B) A CIRCLE OF DYE WAS DRAWN, BUT NOT AROUND THE OTHER. THE PLATE WAS PUT AWAY IN AN INCUBATOR AND AT THE END OF 24 HOURS A PHOTOGRAPH WAS MADE. RIGHT: THE UNRINGED DROPLET (A) HAD NOW SPREAD OVER THE PLATE LIKE A WHITE CLOUD; BUT THE OTHER DROPLET, THOUGH IT CAN BE PLAINLY SEEN THAT THE BACTERIA HAVE GROWN TO A CERTAIN EXTENT THAT PROVES IT ALIVE, COULD NOT PASS THE SURROUNDING RING OF DYE, WHICH ACTED AS A DEADLY BARRIER. NOTICE THAT THE GROWTH OF THE DROPLET (A) ALSO CEASES WHEN THE NEIGHBORHOOD OF THE DYE IS REACHED, THE GERMS TENDING TO KEEP AT A SAFE DISTANCE.

ferent bacteria have been streaked across the plate, three Gram positive bacteria in the center (S, OA, and Su), and two Gram negative bacteria at either end (T and C). The plate has been allowed to stand in an incubator for 24 hours. Both Gram positive and Gram negative organisms have grown on the plain agar (lower portion of the plate). On the gentian violet agar (upper portion of the plate) the Gram negative organisms have grown as well as if no dye were present (T and C); but no growth of the Gram positives has occurred, the dye having completely inhibited their reproduction (S, OA, Su). The selective activity is almost as striking as if one were able, by passing some gas or other into a crowded hall, to kill every brown-eyed person, without affecting those with blue eyes.

This kind of experiment showed that not only might antiseptics be selective as between bacteria and animal cells, but that they might also be selective as between different kinds of bacteria, and that it might eventually prove possible to develop antiseptics suitable to the particular germ responsible for a given infection. An opportunity to test out this idea occurred after the war. Many of the American soldiers, who had had amputations done, developed in their amputation stumps a peculiar disease which often occurs under the conditions obtaining in the war and which is due to the same bacillus as that which causes diphtheria. The bacillus of diphtheria belongs to the Gram positive group of bacteria and is quite susceptible to gentian violet. It proved possible, by the use of gentian violet, at the Walter Reed Hospital in Washington, to free the amputation stumps of the diphtheria bacillus and thus to sterilize them on the principle of what is now known as "selective bacteri-



(*Courtesy of the Journal of Experimental Medicine*)

A B C

PLATE II

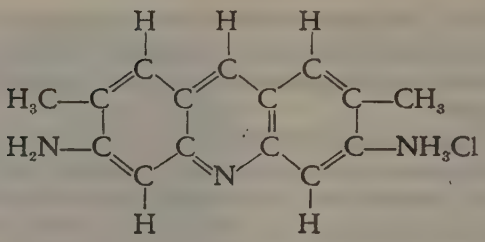
MICROPHOTOGRAPHS OF A MIXTURE OF BACTERIA. TWO TYPES OF GERMS CAN BE PLAINLY RECOGNIZED: (A) THE VERY BLACK, CHAIN-LIKE BROKEN RODS AND THE MUCH SMALLER ELONGATED DOTS, NOT UNLIKE FLY SPECKS. WHEN A MIXTURE OF THESE TWO KINDS OF BACTERIA WAS TREATED WITH A CERTAIN ANILINE DYE AND THEN PLANTED ON AGAR-AGAR. THE ENSUING GROWTH (B) CONSISTED ENTIRELY OF THE BROKEN ROD GERM, THE OTHER (THE "FLY SPECK") HAVING BEEN INHIBITED BY THE DYE. SIMILARLY, USING THE SAME MIXTURE OF BACTERIA BUT A DIFFERENT DYE, THE RESULT (C) WAS REVERSED: THE BROKEN ROD ORGANISM WAS INHIBITED BY THIS DYE AND THE ENSUING GROWTH CONSISTED ENTIRELY OF THE "FLY SPECK" ORGANISM.

ostasis." By "selective bacteriostasis" we mean the use for a given infection of an antiseptic known to have a selective affinity for the particular organism responsible for the infection.

It has also been shown that other dyes have a selective affinity for bacteria which is in some respects the opposite of that of gentian violet, so that if one has a mixture of two different organisms of certain types to deal with one can suppress either of the two desired and obtain the other in pure culture by using one or the other of the two dyes. Microphotographs of bacteria in an experiment illustrating this reverse selective power are shown on Plate II.

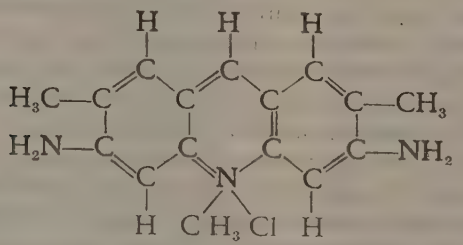
Having demonstrated these two opposite types of selective activity it was an obvious step to attempt to combine them and a dye was devised known as acriviolet which does just this. This is a new kind of synthetic chemical product and both it and gentian violet (as well as brilliant green and other members of the triphenylmethane group) have been used with some success in the treatment of infections.

The Acridine Dyes.—The development of neutral acriflavine—a drug now widely used for antiseptic purposes—from the simpler and less effective acridine yellow with which the experimental study started, illustrates beautifully the marvelous power of chemistry to fashion the molecule to its desires by clipping off or suppressing harmful portions while retaining or enhancing the valuable properties of what remains. In a systematic study of aniline dyes Ehrlich had found acridine yellow to have some therapeutic properties when used against the trypanosomes of African sleeping sickness. Acridine yellow has the structural formula given in I:



I. Acridine Yellow

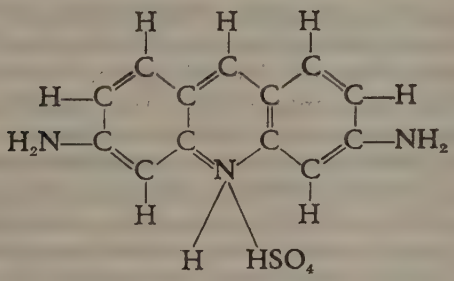
This substance, while of value, was found to need great improvements. Two facts influenced the direction of Ehrlich's researches. The first fact he had, in collaboration with Bechold, discovered early in his work. This was that the introduction of chlorine, or any one of the halogens into disinfecting agents greatly increased their bactericidal powers. The second fact was that the methyl group (CH_3) in a compound works "dystherapeutically", i.e., this group tends to decrease bactericidal power. Ehrlich therefore began by introducing a chlorine radical into the acridine yellow and produced acridinium yellow which has the formula II:



II. Acridinium Yellow

As expected, this change in the molecule resulted in an increase in therapeutic value.

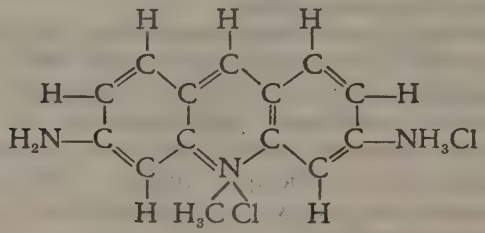
Ehrlich and Benda then attempted to eliminate the (CH_3) groups by chemical manoeuvres which had to be so conducted as always to leave the rest of the molecule essentially unchanged. Elimination of the methyl groups was extremely difficult but once accomplished it confirmed the "dystherapy" theory. The substance now obtained was known as proflavine and it proved to have the formula III:



III. Proflavine

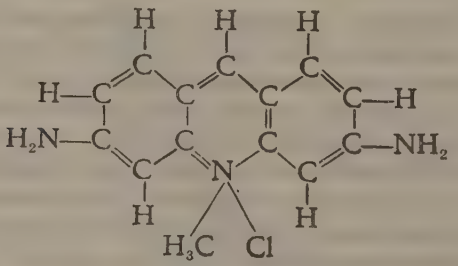
This substance represented great progress over the original acridine yellow but it was still unsatisfactory. Remembering the advantageous effect which the introduction of chlorine radicals into acridine yellow had had, Ehrlich duplicated this procedure, replacing the H and HSO_4 of the above formula with a methyl group (CH_3) and an atom of chlorine. The substance thus produced was found to be quite effective against trypanosomal infections in animals and was called in Germany trypaflavine. In England and America it became known

as acriflavine. It has the formula IV:



IV. Acriflavine

Acriflavine was extensively used, particularly by the British Army, during the war. Hydrochloric acid is easily liberated by the NH_3Cl group, and proved to be a distinct disadvantage. After the Armistice one of the original co-discovers (Benda) set himself to work on the very difficult problems of neutralizing this source of acid in acriflavine without disturbing the acridinium salt. This he succeeded in doing, thus producing an absolutely neutral form of this powerful antiseptic which, even in 1% solutions, will not change the color of Congo Red paper. Neutral acriflavine has the formula:



V. Neutral Acriflavine

The story of neutral acriflavine thus illustrates how, starting with a therapeutic agent of some promise, chemists are able—within limits—so to alter its nature as to render it much more suitable for the desired therapeutic effect. The experimental work necessary to accomplish this purpose is only possible for chemists of the highest training who can bring to the task ingenuity, imaginative powers, and endless patience.

Combinations of Dyes and Metals.—Still another synthetic dye compound has proven of great value. For many years investigators have been trying to avail themselves of the penetrating or carrying power of aniline dyes by attaching to their molecule in a synthetic antiseptic an element like mercury possessing strong bactericidal power but having no capacity of percolating into animal tissue. A number of such compounds have been made, the best known of which is mercurochrome. This is a synthetic product of great value and now widely used. Mercurochrome is a combination of fluorescein and mercury. Fluorescein is a penetrating dye with very little if any bactericidal properties; mercury is a strong antiseptic incapable by itself of reaching beyond the surface. Mercurochrome combines the virtues of the two substances and is a penetrating antiseptic of great importance. The number of possible combinations of this sort is very large and the surface has only been scratched in this field of experimentation. Substances like carbolic acid, which belong in the phenol group, are doubtless capable of similar synthetic combination with dyes; indeed, such combinations are now being worked out. There is no reason why, out of the numerous possibilities in this field, other

compounds may not be devised of great therapeutic value.

Treatment of Blood Poisoning.—One characteristic of the aniline dyes which recent studies have brought to light is of importance. We have spoken of the fact that the more commonly used antiseptics, such as iodine, cannot be injected into the circulation and that in the presence of general infection of the blood (septicemia or blood poisoning) medicine is nearly helpless. This disease is a very serious and often fatal one. Research workers have therefore sought with great eagerness for antiseptic substances which could be safely injected into the blood stream. It has been found that certain of the dyes may be used in this way. Gentian violet and acriflavine have been injected into the blood stream of a good many patients and the procedure, so far as we can now tell, is not attended by great risk. Mercurochrome may also be thus used but the risk with this drug is somewhat greater. Whether the septicemia can be cured by this kind of treatment is as yet doubtful. The matter is now being sharply debated among workers in this field and as there is a certain amount of evidence on both sides of the question, it is impossible to arrive at a conclusion until a large series of very carefully observed injections have been made, along with an equally large series of control experiments. But the lively interest which this investigation has aroused makes it not unlikely that out of the studies which the observations on the aniline dyes have inspired will come success in the treatment of septicemia.

There is no field of medicine in which positive knowledge is more urgently needed, because nowhere is medicine more helpless than in facing this disease. Blood-

poisoning is peculiarly tragic in its possibilities because of its propensity to affect the young and vigorous. It may arise from a simple and apparently innocent lesion —like a pimple on a baby's lip or chin, or a slight infection about a hangnail on a child's hand. In many cases every effort to check the disease is unavailing; advancing with appalling rapidity, it terminates fatally in a few short days. It is altogether likely that an internal antiseptic which proved effective against septicemia would also be of fundamental value in the treatment of endocarditis, or infection of the valves of the heart. This is another disease which often picks out the young for its victims. It frequently terminates fatally, and even when this is not the case, it is likely to leave the patient badly handicapped. In the majority of cases, little can be accomplished by present day treatment.

Internal Antisepsis.—Physicians have also long been anxious for what is called an internal antiseptic; that is to say, an antiseptic which can be administered by mouth and whose antiseptic properties are not destroyed by the processes of digestion, absorption and excretion. Many of the commonly used antiseptics are too toxic or too irritant to be taken by mouth; and others are so changed after administration that they lose their efficiency. A few substances are known, like hexamethylenetetramine (introduced as urotropin), a combination of formaldehyde and ammonia, which after being swallowed and absorbed pass into the blood and appear in excretions, as the urine, in sufficient quantity to inhibit the growth of bacteria. But they have not as a rule proven very satisfactory.

In 1921 Johnson and Hodge showed that the lower compounds in the series of the alkyl resorcinols, $(C_nH_{2n+1}).C_6H_3(OH)_2$, possessed a high degree of bactericidal power and that they were also relatively non-toxic. Experiments indicated that it was the alkylation of the resorcinol which increased its bactericidal powers and decreased its toxicity in direct proportion to the sum of the atomic weights of the atoms of the alkyl chain C_nH_{2n+1}. The peak of bactericidal power was reached in hexylresorcinol, $C_6H_{13}.C_6H_3(OH)_2$, a substance which could be administered by mouth in doses sufficient—when excreted in the urine—to render it bactericidal. This substance—another example of chemical manipulation of the molecule to meet particular needs—appears to possess the following desirable qualifications of an internal antiseptic: (1) It is chemically stable. (2) It is non-toxic in highly effective doses. (3) It is non-irritating to the urinary tract. (4) It is bactericidal in high dilution in urine of any reaction. (5) It is eliminated in high percentage by the kidney, largely in an inert combination with some constituent of the blood, but unchanged in sufficient amount to impart definite bactericidal properties to the urine.

The Lure of Chemotherapy.—It is clear from what we have said that synthetic chemistry is proving a valuable ally in the battle which medicine is waging with pus infections, whether localized, spreading, or general. What attraction does this field offer to the young chemist interested in experimental investigation?

It offers first of all the lure of one of the most fas-

cinating of all sciences—the science of chemistry itself. And the particular kind of chemistry into which the worker in the chemotherapeutic field will be drawn is one of the most interesting branches of this science, for it is a branch concerned with the analysis of therapeutic products and their reconstruction by synthetic means along the lines suggested by creative ingenuity.

It offers, in addition to the lure of chemistry, the lure of bacteriology. Pursuit of this infinitely complex science keeps the worker in close contact with the microbiological world, a world that teems with wonders, a world alive with fascinating puzzles, a world on whose activities the very existence of man depends.

It offers, in the third place, the lure of medicine itself. Workers in chemotherapy must form a close acquaintance with all the absorbing problems connected with the physiology and pathology of the human body. They will come into constant contact with the immensely interesting manifestations of disease. They will have to ponder over the manifold complexities of therapeutics.

And to those investigators whose imagination is capable of being touched by the sufferings of humanity, whose hearts would glow with gratification if their scientific endeavors could contribute to the amelioration of the human lot—let it be remembered that one of the greatest of all scientists, Pasteur, was a man of this type. To scientific investigators of a kindly and sympathetic calibre, synthetic chemistry ought to make a strong appeal.

Chapter IX—Article 4

THE BATTLE AGAINST MALARIA

C. C. BASS, M.D.

Professor of Experimental Medicine, School of Medicine, Tulane University

Dr. Bass dates his connection with the medical school of Tulane University from 1905. He has been Professor of Experimental Medicine since 1912 and Dean of the School of Medicine since 1922. In 1912 he was head of the Tulane Expedition to Panama for the Study of Malaria. He has made important contributions also in the study of other infectious diseases.

Man has been a victim of malaria since pre-historic times, and there is every reason to believe that this disease was one of his enemies for ages before the dawn of civilization. In the fifth century B.C. the father of medicine, Hippocrates, gave accurate descriptions of the disease. He differentiated what we now know as malarial fevers from the continuous fevers and divided them into quotidian, tertian and quarton, according to the frequency of the paroxysms. His description of these intermittent fevers could coincide with no other disease than malaria. The following paragraph is quoted from his treatise on "Airs, Waters, and Places:"

"Those who dwell in hollow, hot districts where the winds and water are warm are neither tall nor straight. If they drink the water of the place they have diseases of the spleen and stomach. They are stout and fleshy, dark-colored and bilious. By nature they are neither courageous nor of great powers of endurance. But there are no ill effects when the water is drained off."

Ancient Greek literature from the time of Hippocrates, over 400 years B.C., to the fourteenth century

A.D., describes the disease in almost precisely the same terms and with exactly the same symptoms as we meet today. This, with much other data, is abundant evidence that malaria was rife during the height and the decline of Greek civilization.

As evidence that malaria played an important rôle in public affairs at the time of the Roman Empire, Mannaberg quotes from a letter from Horace to Maecenas as follows:

"Horace begs Maecenas to extend his leave of absence, so as to permit him to remain away during the intensest of the summer heat, for when the first figs ripen and faces become pallid from fever, the chief of the funeral pomps, with his black assistants, is very active, and the reading of wills becomes the order of the day."

Cinchona Bark and Quinine.—A new epoch in the history of malaria began with the discovery and introduction into Europe of cinchona, about 1640. Cinchona came at a time when the little knowledge about diseases, their causes and treatment, was so beclouded and overshadowed by ignorance, superstition, and charlatanry, that the remedy was accepted and became available for extensive use only a great many years later. Gradually its action was recognized. Torti, in 1753, first applied the term "malaria" to the disease in line with the belief long held by himself and others, that it was due to bad air. He divided fevers into those that yielded to the bark, which he called malaria, and those that did not. Thus cinchona not only provided man with a remedy with which the disease could be treated and cured but also with a valuable instrument for differentiating it from other fevers. The therapeutic test

is employed in practice to a large extent even now. While another more accurate method, examination of the blood for the malarial parasites, is available to all well-equipped practitioners, there are many others who, in practice, rely upon the therapeutic test. It may be laid down as a rule with few or no exceptions, that a case of fever or chills and fever that continues for more than three or four days while the patient is taking a proper amount of quinine, is not due to malaria; but, it cannot be said with any degree of accuracy that cases of fever that do stop while the patient is taking quinine are due to malaria.

Accuracy in diagnosis and treatment of malaria was promoted by the discovery of quinine in the cinchona bark and its isolation by Pelletier and Coventou in 1820. The bark varies greatly in its content of the anti-malarial alkaloids and as long as it was necessary to use it, inaccuracy and guess work necessarily prevailed. Discovery of quinine was the first great contribution of chemistry to man's constant warfare against malaria which, as we have already stated, dates back to prehistoric times.

The Malaria Parasite and Its Transmission.—The battle was fought against an invisible and mysterious foe until the malaria plasmodium was discovered by Charles Louis Alphonse Laveran, a French army surgeon, in 1880. Through this discovery Laveran became one of the greatest benefactors to mankind of all times. From that time on it has been possible to make accurate diagnosis and to clarify and greatly expand the previous more or less confused knowledge about the disease. The study of malaria was greatly stimulated under the new impetus of this discovery. Accurate

knowledge was acquired much more rapidly, and with it man's weapons against the disease have been greatly multiplied.

But another fundamental fact was still to be uncovered, viz., the mode of transmission from man to man. Credit for this goes to Sir Ronald Ross, who definitely incriminated the mosquito in 1898. This discovery was soon confirmed throughout most civilized countries where malaria prevails. The evidence was soon overwhelming and within only a few years it was quite generally accepted that malaria is carried from man to man by mosquitoes. It took considerably longer time to learn and to inform the world in a convincing way that the disease is carried by mosquitoes only, and what is still more difficult to understand, only by certain of the species of mosquitoes that attack man. Even today there are many people who accept the mosquito transmission of malaria with a certain amount of reservation or do not accept it at all. Although this is largely among the more ignorant population, unfortunately their numbers are so great as to retain the disease as an important factor in the health of many parts of the civilized countries of the world.

In addition to the discovery of these fundamental facts, increments to our knowledge have been added during the past thirty years by a large number of scientific workers until most of the facts concerning the causation, prevention, and cure of malaria are now well known.

Mosquitoes.—Discovery that malaria is carried from man to man only by certain species of mosquitoes placed at our command another most powerful weapon in man's battle against the disease, viz., prevention of mos-

quitoes. Fortunately the effective flight range of the kind of mosquitoes that transmit malaria is not great, practically not more than from one-half to one mile. These mosquitoes bite mostly at night. The larval stage in the life of the insect is passed in water, usually under suitable cover of vegetation for shade and protection. Where there are no favorable collections of water there can be no mosquito breeding. Draining or filling wherever practical to prevent collections of water in which mosquitoes could breed, eliminates breeding, if thoroughly done.

Breeding may be controlled also by application of oil to the breeding pools at sufficiently frequent intervals, usually by spraying, thereby suffocating the larvae which are prevented from coming to the surface for air. More recently some use has been made, with considerable success, of dusting over the water very small quantities of poisonous chemicals such, for instance, as Paris green. The larvae which feed at the surface swallow minute particles of the poison dust floating on the water and are killed. There has been considerable success with poison dust spread over swamps and large areas where drainage would be entirely too expensive to be practical.

During the past twenty years, particularly during the past ten or twelve years, there have been conducted hundreds of successful malaria control projects in different parts of the world based largely and sometimes entirely upon control or elimination of mosquito breeding from the area. These projects have demonstrated over and over again the possibility and feasibility of complete suppression of the spread of malaria by suppressing mosquito breeding.

Mosquitoes do not have malaria parasites in them until they get them from the blood of a man who has

malaria, and a man does not have malaria until he is inoculated with the parasites by an infected mosquito. It is a continuous cycle or an endless chain of transmission by which the parasite is propagated from man to mosquito, and from mosquito to man. The parasite is capable of reproducing or multiplying in each of these two hosts. But if there are no mosquitoes, no transmission can take place and the infection is terminated in those persons who have the disease, sooner or later, either by spontaneous recovery, as a result of treatment, or sometimes by death. No argument should be required to convince any intelligent thinking person who knows these facts, that elimination of mosquito breeding will also eliminate malaria. Neither should demonstrations be required, but if so, they are plentiful. The largest demonstration of malaria control, chiefly by mosquito control, in the western hemisphere, was made in the building of the Panama Canal. The French attempted to build the canal and failed. They left fifty thousand tombstones and mountains of abandoned machinery and equipment as the most convincing evidence of the supremacy of malaria and yellow fever over man at that time. Building of the canal later by the United States was made possible by the control of these diseases under the leadership of the immortal Gorgas. The site of the Panama Canal would remain in forest and jungle today but for our knowledge of the cause and control of malaria. The battle was won against this formidable foe, and this, with many other equally successful battles in other places, has long since proven to all the world that malaria can be conquered anywhere by the intelligent expenditure of the necessary effort and cost.

Any town, city, community, or district can get rid of malaria in short order if the will is there and if the necessary resources are available. Many have been transformed almost like magic during short periods of only a year or two from malaria hotbeds practically to health resorts, so far as malaria is concerned. It is not necessary to cite instances here, for they are so numerous that everyone knows about some of them.

However, while intensive malaria control by controlling mosquito breeding can be made almost one hundred per cent effective, the limitation of cost has made it impossible to apply the method thus far to whole countries or very large areas. As a matter of fact the total area in this country in which such measures have been applied is almost negligible as compared with the area in which malaria has prevailed. The number of people reached is too small for this to have materially reduced the prevalence of malaria in the country as a whole.

Malaria is rapidly decreasing both in frequency and in severity in the country. The rate of decrease has greatly accelerated during the past ten or fifteen years as a result largely of the greatly increased activities against malaria, of the United States Public Health Service, The International Health Board, State Boards of Health, county health units and other agencies. Besides these, as a result of education, individual families and groups have taken greater precautions against the attacks of mosquitoes and against the disease. By certain propaganda and educational efforts the practicing physicians of the country have been led to devote more attention than formerly to the cure of the infection in the cases of malaria they treat. Formerly and even now, too many

malaria patients treated by physicians were discharged after relief from the active symptoms of malaria, still infected, without adequate treatment to cure the infection. Such patients are malaria carriers, potential sources of infection, through mosquitoes, to others. On the other hand every such patient finally cured of his infection is one less source of infection to others. If all the people who have malaria could be cured of their infection, there would be no further transmission because there would be no malaria to transmit.

The Decline of Malaria.—Advancing civilization and development of a district or region generally carries with it a number of conditions that tend to cut down and sometimes to completely eliminate malaria. Clearing and draining the land for agricultural and other purposes and increased prosperity, providing better treatment with quinine, are most important factors. These influences have led to the decline and disappearance of malaria from large areas of whole countries in different parts of the world, even before the cause or mode of transmission were known.

In 1697 Morton described accurately the malarial fevers that prevailed in London, and in England and Wales at that time. Sydenham in 1726 gave a most complete description of the intermittent fevers prevalent throughout England and parts of Scotland, the infection continuing in England until 25 to 50 years ago. By that time it had disappeared before it was known that mosquitoes transmitted it. Holland was almost as malarious at the beginning of the nineteenth century as the famous Agra Romana; now the infection is strictly focal and of

mild character. A hundred years ago Minnesota, Michigan, and the region around the Great Lakes were intensely malarious. Malaria was very prevalent in New York, Philadelphia and Baltimore and the surrounding country during the first half of the nineteenth century, but it had almost disappeared by the beginning of the twentieth century. Dr. L. D. Frichs, Director of Malarial Investigations by the U. S. Public Health Service, states that "During the past fifty years the area of endemic malaria in the United States has shrunk to less than one-third of its former extent without any effort on the part of the health authorities to control the disease. At the time of the Civil War malaria was the largest single cause of death in the Southern States, hemoglobinuria and coma being common. During the World War it was a negligible factor in the health of the very much larger number of soldiers in service."

Malaria is slowly but surely being suppressed in most of the highly civilized countries of the world. In this country it is coming to be of little importance except in small areas where circumstances and conditions have favored the disease more and man less. We are winning the battle. Unfortunately the outlook is not so favorable for the less well civilized and less fortunately situated people of the world. The proportion of the people in many countries who have the disease varies from none in some countries to practically 100 per cent in others. Even now millions, yes, more than a hundred million people throughout the world, suffer with it every year and more than a million die.

Contributions by Chemistry.—In conclusion it may

be of interest to point out some of the important contributions that chemistry has made to the success that has already been attained and assured in man's warfare against one of his greatest disease enemies.

Chemistry gave us quinine from cinchona bark, permitting of accuracy in treatment (and therapeutic diagnosis before the discovery of the parasite) with which all except moribund cases can be cured of their symptoms and of their infection, thereby preventing deaths and spread of the disease.

Chemistry gave us the aniline dyes and other chemicals widely used in present day methods of diagnosing the disease.

Chemistry has been of inestimable value in examination of the blood and urine in studying the absorption and elimination of quinine, thus permitting of accuracy in dosage and methods of administration.

Chemistry has provided poison powders which have been found of much use in economical control of mosquito breeding.

Chemistry has provided a number of synthetic preparations which have been used as remedies against the disease. While none of these, so far tried, are better than, or even as good as, quinine, there is good reason to hope that one will be discovered that will be far superior. Although quinine is an effective remedy, it has serious drawbacks, chief of which is causation of considerable discomfort to many people who take it and the long time (6 to 8 weeks or longer) necessary to effect a cure. The greatest need today is for a quicker acting, harmless specific, and we must look to chemistry for it.

CHAPTER IX—ARTICLE 5

THE ADVANCE AGAINST AMEBIC DYSENTERY

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Dr. Geiger has served as assistant director and acting director of the California State Board of Health (1913-16), as an epidemiologist of the U. S. Public Health Service (1916-24), and as assistant commissioner and then executive officer of the Department of Health of the City of Chicago (1924-28). During these years he also was an Assistant Professor of Hygiene at the University of California (1913-16) and associated with the University of Chicago (1922-28) as incumbent of a chair on epidemiology. Recently Dr. Geiger has accepted a professorship in the Hooper Foundation of Medical Research of the University of California. His research work has covered such important fields as botulism, malaria, typhoid and amebic dysentery.

Dr. Williamson's medical training was secured both in this country and abroad. He has been connected with the Medical School of the University of Illinois since 1902 and since 1914 he has been the head of its Department of Medicine. He served in the war as Lieutenant-Colonel in the Medical Corps, U. S. A., and in 1918 received the gold medal of the American Medical Association. His research work in medicine has been wide and varied.

Miss Kaplan is a graduate of the University of Chicago and received the M. S. degree in Hygiene and Bacteriology in 1921. Since then she has specialized in medical laboratory research, especially also in the field of amebic dysentery.

Many medical men are under the impression that amebic dysentery is a strictly tropical disease. While it is endemic in many tropical and subtropical regions it is by no means limited to those localities.

Historical Introduction.—The first observation of the presence of amebae in a case of dysentery was made

by Loesch in 1875 in the instance of a patient suffering from dysentery in Russia. This discovery initiated a long series of investigations, lasting some 40 years, by a large number of workers. Important as their contributions were in preparing the way for a final complete elucidation of the problem of the disease, a great deal of confusion and delay in the proper treatment of the disease resulted from their failure to realize: (1) that dysentery is a symptom of several different pathological conditions with no one specific cause for all of them; (2) that man harbors a number of different species of amebae, only one of which, Entameba histolytica, is pathogenic. It is now known that epidemic dysentery is due to dysentery bacilli. It is now equally clear that a certain type of dysentery is due to Entameba histolytica and that unrecognized, this kind of dysentery is not only a menace to the life of a sufferer from the disease, but also a source of danger to whole communities.

During and immediately following the World War great anxiety was expressed in medical and zoological circles in England and in this country that the return of the soldiers from Europe to the United States and from the Mediterranean countries and Egypt to England might result in an increase of carriers of amebic dysentery with a subsequent increase of clinical cases. Protozoan surveys, started in England by Carter, Mackinnon, Mathews, Smith and Dobell showed that the rate of infection with Entameba histolytica and other intestinal protozoa was somewhat higher in those who had been out of England than in those who were never away from England. This increase was due to travel in Egypt, Mesopotamia, etc.

In post-war American surveys, Kofoid found a

greater percentage of carriers of Entameba histolytica than was found for English dysenteric and non-dysenteric patients from war areas about the Mediterranean. The work of Drs. Charles Wardell Stiles and William E. Boeck of the Hygienic Laboratory, on the other hand, showed that the rate of infection with Entameba histolytica on overseas and home service men was approximately the same. The latter workers found that their results compared favorably with those of the English workers. The results of Drs. Stiles and Boeck show that the rate of infection for Entameba histolytica in institutions is higher because institutional life, such as is found in asylums and corrective institutions, is conducive to the spread of protozoan infections. Their results also show that the highest rate of infection was at the United States Public Health Hospital No. 43 on Ellis Island, New York, chiefly among immigrants from Roumania, Italy and Greece. It is also found along the Mexican border and in Louisiana, Mississippi, etc., but it is by no means limited to those states.

Identification of Entameba Histolytica.—As stated before, while man may harbor a number of different species of amebae, amebic dysentery is caused by only one of these, Entameba histolytica. The motile forms of this ameba as seen in warm freshly obtained specimens from active cases of amebic dysentery, are 25 to 40 microns* in size; they move very rapidly and ingest red blood cells. Occasionally, as many as 50 or 60 red blood cells may be seen in a single parasite. The nucleus of the ameba is usually indistinct during the actively motile stage, but when it is visible it can be readily

*A micron is the millionth part of a meter.

differentiated from the red blood cells by its larger size, its colorless appearance, a thin ring of granular chromatin, material arranged around the inner wall of the nucleus, and the very small centrally placed karyosome. In a fresh smear the nucleus is colorless while the chromatin material and karyosome have a refractile greenish tint. The red blood cells have a greenish yellow tint.

Clinical symptoms of amebic dysentery are produced by these active forms. The ulcers are usually limited to the large bowel, but should treatment be neglected, the ulcers might perforate and produce peritonitis. The organisms may gain their way to the liver, lung and brain and produce abscesses. Secondary bacterial infection may also take place in the large bowel resulting in abscesses.

Immediately or shortly after treatment is instituted, the symptoms abate, diarrhea ceases, the organisms disintegrate or round off. At this stage one may encounter some difficulty in recognizing them and may have to resort to making permanent slides according to Schaudinn's method and search for the typical Entameba histolytica nucleus with an oil immersion lens. As treatment is continued the stools become well formed and one may be unable to find any organisms at all or, occasionally, encysted forms may be found. These encysted forms vary in size from 6.6 to 15 microns in diameter. They have from 1 to 4 nuclei with very small centrally placed karyosomes. During the early stages chromatoid bodies (rod-like structures) may also be seen; but as the encysted forms become older, these bodies are absorbed.

For the identification of the encysted forms, a strong iodine solution is very satisfactory. With this solution

the walls of the cysts and nuclei stain dark brown, the cytoplasm pale brown, the walls of the chromatoid bodies medium brown and the karyosomes appear as refractile bodies.

It is very important to make a correct diagnosis of cases as well as of carriers because: (1) Failure to give specific treatment may result fatally. (2) Every carrier represents a focus of infection that is liable to spread the disease. (3) On the other hand, a person free from amebic dysentery isolated with a group of convalescents may contract the disease. (4) Isolating a person who is free from amebic dysentery and is not a carrier of encysted Entameba histolytica results in economic loss to the person involved and disruption of a system where that person is employed.

Stools of persons with amebic dysentery can often be recognized macroscopically as well as microscopically. Amebic dysentery stools are bloody, mucoid, free from macrophages and pus unless the case be of long standing, when secondary infections have set in. On the other hand, stools showing a great deal of pus, rich in cellular exudate, some mucus and only now and then streaks of blood should be looked upon with suspicion as being bacillary in nature. In all such cases bacteriological examinations for dysentery bacilli should be made without delay. Failure to isolate dysentery bacilli in cases of long standing does not rule out bacillary dysentery.

Extreme care should be exercised in examining fresh specimens (obtained preferably with a rectal tube or proctoscopically) for amebae. This should be done immediately upon discovery of the symptoms and *before* treatment of the patient. After specific treatment has

been given, the organisms, as stated before, begin to disintegrate and it is difficult or even impossible to recognize the organisms. Occasionally strained mounted preparations are of assistance. All convalescents of amebic dysentery should be examined repeatedly for encysted Entameba histolytica. Periodic examinations should be made after they are discharged from the hospital. Without these precautions, they are likely to be carriers and a serious source of danger to the community, as we shall see presently.

TREATMENT

Chemical Warfare on the Entamebae Histolytica.—The methods of treatment of amebic dysentery vary with individual physicians but all methods depend on the use of specific chemicals intended to dislodge and destroy the enemy. It is particularly fortunate that chemistry has placed at the service of medicine a variety of agents, since it has been found that under certain circumstances the infesting amebae, surviving a first attack by a chemical, may become immune to that chemical. As a result, the very first treatment is as concentrated and powerful a one as the condition of the patient permits, and it is best followed up by a change to a new specific chemical which is also a poison for the amebae.

The following are standard drugs and accepted methods of treatment:

Ipecac.—This drug was introduced to Europe in 1682 by Piso, a traveler who heard of its beneficial results during his sojourn in Brazil where it had been used for many decades by the natives against varied enteric disturbances. Later, Helvetius, a Dutch physician

residing in Paris, procured large quantities of it. The dauphin, son of Louis XIV, who suffered from dysentery for many years, was relieved by the use of ipecac.

Ipecac, like other vegetable drugs, such as cinchona bark, belladonna, etc., owes its effectiveness to the presence of specific chemical principles. It was used for many years, like cinchona bark, before these alkaloids were isolated; but unlike cinchona bark, ipecac is still widely used now, even after its alkaloids have been isolated and made available in pure form. Rio ipecac is considered to possess the highest content of alkaloids. It is put up in pills of 5 grain quantities coated with salol, which prevents solution of the drug in the stomach, where it would be likely to cause vomiting; in the alkaline intestinal tract, the salol coating is dissolved and the ipecac is able to deliver its attack in concentrated form.

A full course includes the retention of at least 100 pills, equivalent to 500 grains of the powdered root. Efficacy of the treatment depends upon the concentration of full strength within the large bowel.

Emetine.—In 1817 Pelletier and Magendie, French chemists, studied the constituents of ipecac root and isolated an alkaloidal substance from the plant. In 1894, and later, Paul and Crowley by more refined analysis proved conclusively that the "emetine" of Pelletier and of many later investigators was composed of three different alkaloids. For one of these they preserved the name emetine and the term is now limited to this single chemical principle; its chemical formula, $C_{30}H_{44}N_2O_4$, shows how complex is its molecule. It is the most active of the three alkaloids in the destructive war on Entameba histolytica and, as we shall see presently, its sep-

aration as a pure principle by chemical analysis has made it available for intravenous use by medicine. The other two alkaloids found in ipecac received the names cephaeline and psychotrine. Weld in 1895 showed that the action of ipecac in producing vomiting is due to the alkaloid cephaeline and that pure emetine and psychotrine have no emetic action.

Emetine is used in the treatment of amebic dysentery in the form of its hydrochloride or hydrobromide salt. The majority of physicians prefer emetine hydrochloride. The treatment depends upon the condition of the patient. In highly acute, fulminating cases intravenous injections may be resorted to. In chronic cases a hypodermic injection in the morning followed by a smaller dose by mouth at night is continued for ten to twelve days. Some give emetine hydrochloride with Alcresta Ipecac (salol coated) which is not dissolved in the stomach.

The dosage must be guarded carefully because too little at a time may accustom the Amebae histolytica to the action of the drug and result in a state of immunity where the patient becomes "emetine-fast"; too much may result in such serious effects as depression and paralysis of the heart, gastro-intestinal irritation, acidosis, peripheral neuritis, etc. There is definite danger to people with disturbed circulatory, digestive and metabolic systems.

Bismuth Preparations.—Bismuth subnitrate has been used for many years as an internal "dusting powder," coating the mucous membranes and acting as a mechanical protection. It has been recommended for use in the treatment of amebic dysentery. W. E. Deeks working in Ancon Hospital in the Canal Zone found in 1908

that it is necessary to give it in very large doses, best suspended in a tumbler of plain or effervescent water or in milk every 3 to 4 hours during the day for one or two weeks.

A combination of emetine and bismuth in the form of emetine bismuth iodide is a more modern preparation. On account of its limited solubility it is less likely to cause vomiting than emetine hydrochloride when taken by mouth, and it is said to have given good results in the treatment of chronic cases and carriers of amebic dysentery even when hypodermic administration of emetine alone had failed.

Arsenicals.—Arsphenamine and neoarsphenamine, the specific remedies for syphilitic infection, have been used with some success in known amebic dysentery. But a simpler arsenical, closely related to arsphenamine, has been said to have particularly favorable effects in the treatment of amebic dysentery. This synthetic drug is known as acetarsone (also as stovarsol) and has the structure expressed by the formula:

$$CH_3CONH.C_6H_3.(OH)AsO(OH)_2$$

It is said to yield satisfactory results both in the eradication of dysenteric cysts (found especially also in carriers) and for general amebic dysentery.

Chaparro Amaegosa.—Particularly interesting as a remedy for amebic dysentery is a drug known as chaparro amaegosa. The plant itself is a thorny bitter bush belonging to the Simarubaceae, growing about 4 feet in height on dry rocky hilly sections of Northern Mexico and Southwestern Texas. It has been used for many years as a domestic remedy in Mexico and Southern

Texas. Zachary Taylor's soldiers used it during the Mexican War. Putegnat of Brownsville, Texas, 1883, was among the first to use it advisedly in the treatment of dysentery. Later other Texan physicians published articles advocating its use. The drug is employed as an infusion or fluid extract. The infusion is obtained by boiling all parts of the shrub—roots, branches, leaves and red berries—with water for thirty to forty-five minutes; the infusion is strained for use. The treatment is continued until the patient is relieved of symptoms. The drug has a constipating effect and salines may be given. The infusion may also be given per rectum in amounts according to what the patient can retain 15 to 20 minutes.

The fluid extract is also frequently used in place of the infusion.

Other Measures.—Quinine irrigations of the large bowel of increasing strength (up to 1 to 500) are beneficial, as are also silver nitrate irrigations (increasing to 1 to 500).

Copper sulphate, potassium permanganate, hydrogen peroxide, thymol, coal, oil, etc. are also used. The chief virtue of these is cleansing the bowel of retained mucus and inflammatory debris which might interfere with healing.

An illustration of a typical mode of treatment of acute and subacute cases is as follows: Rest in bed; liquid diet—hot and in small quantities; a saline purge; some opiate—preferably morphine, hypodermically in small and frequently repeated doses; emetine hydrochloride, subcutaneously in the morning, by mouth at night, for 10 to 12 days; quinine irrigations.

Failing to clear up after one series of treatments, the

treatment may be repeated after a short period of rest, or, if there is danger of ill effects from too much emetine, the patient may be given chaparro amaegosa or stovarsol.

Carriers are treated in the same manner with the exception that greater stress is placed on the flushings.

The outcome depends upon the period when the patient comes for observation and on thoroughness of specific treatment. In acute cases emetine should be used subcutaneously or intravenously. The chances for recovery are lessened by hepatic, lung or cerebral abcesses.

A STUDY OF A TYPICAL FOCUS OF INFECTION

Repeated cases of amebic dysentery have come to our attention in Chicago in the last few years. That the disease is not as uncommon as many people believe it to be, may be shown by the fact that at least 35 cases of amebic dysentery and two or three cases of amebic colitis have been treated at the Cook County Hospital of Chicago during the last year. Most likely the reasons that few medical men had encountered amebae in diarrheal and colitic stools have been due to their failure to examine the stools, to inadequate material or to inefficient laboratory examinations. The success of positive findings depends upon thorough examination of fresh material (preferably obtained with a rectal tube) and on careful and repeated examinations of bloody mucoid diarrheas for living forms, and convalescents for encysted forms.

The immediate stimulus for the present survey was the simultaneous appearance of a number (six or seven) of cases of amebic dysentery (simultaneously or within short intervals) in one large hotel in Chicago. The disease was not confined to any particular group or

nationality, but appeared in two or three guests, two housemen, a janitor, a cook and an officer who was stationed there during that period. The last case of amebic dysentery which came to the Cook County Hospital a short time ago, gave a history of working in that place in the Winter. Shortly, three other cases, who worked at the same place during that time, came to our attention—two housemen and a maid—giving a total of 10 active cases.

The simultaneous occurrence of so many cases of amebic dysentery in a single place under the climatic conditions found in Chicago led to the suspicion of the existence of a carrier of amebic dysentery among the food handlers. Through the courtesy of the manager of the house permission was granted to examine the kitchen help, cooks, bakers, fruit and vegetable handlers, pastry cooks, ice-cream makers, dishwashers, pantry help, etc.

The work was done right at the place: A room with a bath was obtained; specimens were collected and examined immediately, one person at a time. The greatest care was exercised in making the microscopic examination for worms, ova, and intestinal protozoa, with the special view of finding Entameba histolytica, cysts as well as active forms, if present. The work was done on the basis of two examinations for each person.

Our survey at the place where the cases of amebic dysentery occurred resulted in the uncovering of one acute case and of two carriers of Entameba histolytica. Of the carriers one was a pantry girl and the other a vegetable girl. The active clinical case was a night cook (an Assyrian) who was ill with amebic dysentery for a number of weeks and had been treated by an Assyrian

doctor who failed to report the case. This cook being on night duty, when the work is not very heavy, was able to pursue his duties most of the time, having stayed out only two or three days with an excuse that he was suffering with "stomach trouble."

We cannot state definitely which of these three persons was the source of infection. We know from our own experiments and that of the English workers, Walker, Sellards and Dobell, that the living forms do not represent the immediately infecting forms, but that they produce the clinical symptoms of amebic dysentery. The encysted forms are the transmittable ones. Whether the cook had been a healthy carrier prior to developing his clinical symptoms or whether he as well as the other clinical amebic dysenteries were victims of one or both of the carriers of encysted Entameba histolytica, cannot be ascertained. Since then we have not had another case of amebic dysentery in that place.

O'Connor and Wenyon, in their work in Egypt, found the largest number of carriers of amebic dysentery among food handlers and prisoners. This may be explained on the basis of unsanitary conditions of the soil as well as of the institutions. Food handlers working with vegetables coming from contaminated soil, become infected, after which dissemination is very easy by flies as well as careless workers. With our food handlers conditions are different; most if not all of our vegetables come from soil free from amebae, but should there be one carrier in a kitchen, the means of dissemination are quite ready.

Most of the cases at the Cook County Hospital gave a history of having worked on railroads up North or in the South, using stagnant water for drinking pur-

poses. One case worked at the Stock Yards; two cases were ex-sailors—one from Egypt and the other had been in India. Several cases stated that they had never been outside of the city.

Results of the Survey of the Food Handlers.—A survey was made of 1148 food handlers in Chicago with the following results:

Types of Protozoa	*Number of Cases*	*Percentage*
Entameba coli	220	19.16
Entameba histolytica	27	2.35
Entameba histolytica	2	(clinical cases)
Endohmax nana	23	2.00
Iodamoeba butschlii	61	5.31
Giardia intestinatis	75	6.53
Trichomonas intestinatis	21	1.82
Chilomastix mesnili	22	1.91

The distribution of the carriers of Entameba histolytica was as follows: three cold meat handlers, one head night baker, one baker, one buffet man, five cooks, one fry cook, one vegetable salad girl, five fruit pantry girls, one girl who made waffles, one colored steward's assistant, three dish washers, one girl who prepared toast, and three pastry men.

Of the active cases, one was an assistant night chef and the other, a coffee man. This coffee man gave a history of coming north from Louisiana about two and one-half years ago and having had amebic dysentery ever since in spite of periodic treatment. He worked in three or four different places as houseman, silver polisher and finally as coffee man when we discovered him.

The number of flagellates, worms and ova might have

been increased by administering salts, because most of the specimens obtained were from the lower bowel, the region of the amebae, but most worms and flagellates are found in the small intestines. The important point at issue was the uncovering of amebic cases and carriers. All of the cases of amebic infections as well as the case of hookworm were removed from the kitchen immediately and sent to the hospital for treatment.

Comment.—The results of various workers, as well as our own work, indicate that amebic dysentery is not limited to the tropics and subtropics. That the present state of increase of cases of amebic dysentery may be due to a number of factors, such as the greater stress placed on medical zoology in college curricula, improved clinical laboratory examinations, the reporting of cases acting as a stimulus towards more thorough examinations of diarrheic stools. Increased migration from the southern to the northern states may well be another factor.

Conclusions.—

1. Amebic dysentery is not limited to the tropics and subtropics.

2. Careful examinations of freshly obtained specimens should be made on all diarrheas and cases of colitis showing bloody mucus.

3. In post-diarrheal stages diagnosis can be made readily by finding encysted Entameba histolytica.

4. The incidence of carriers of Entameba histolytica in large groups of food handlers should probably be investigated as an epidemiologic procedure.

5. Specific chemical treatment is effective when it is promptly and carefully employed. Ipecac and its most important active principle, emetine, as well as bismuth subnitrate, emetine-bismuthous iodide, acetarsone

(stovarsol) and chaparra amaegosa represent a powerful chemical armament for the overthrow of the Entameba histolytica. They place at the disposal of medicine not only effective weapons for a single siege but by their variety make it possible to push the attack to a successful issue even when the amebae have become immune to a specific used first in too weak an attack on the enemy.

NOTE. The literature on intestinal protozoa and worms has been reviewed very carefully by William C. Boeck and Charles Wardell Stiles (Hygienic Laboratory Bulletin No. 133, Studies on Various Intestinal Protozoa, Especially Ameba of Man); by C. M. Wenyon and F. W. O'Connor (Human Intestinal Protozoa in the Near East); and by Dobell (The Amebae Living in Man).

Chapter IX—Article 6

THE HOPE OF THE LEPER

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Dr. Adams started his career as instructor of organic chemistry at Harvard University (1913-16). Called to the University of Illinois in 1916, he advanced rapidly to a professorship (1919) and in 1926 became head of the department of chemistry. He served as Major in the Chemical Warfare Service. In 1927 he received the Nichols Gold Medal from the American Chemical Society for his important work on chaulmoogra oil. He is the inventor, with Dr. Oliver Kamm, of butyn, a local anesthetic superior to procaine for use on mucous membranes, and is one of our leading scientists in the field of organic chemistry.

Leprosy is a disease which has attracted attention from the earliest times in history. There are frequent references to it in the Bible, Talmud and Koran. In the Old Testament, Exodus IV, there is the incident of Moses, commanded by the Lord to put his hand in his bosom and when he drew it forth it was as leprous as snow. In Leviticus the disease is referred to as "the plague of leprosy," a "fretting leprosy." It is called "unclean." The horrors of it were well known, and garments and linen were ordered burned as early attempts at disease control. Though its importance was realized, the descriptions of it are inaccurate and incomplete and it was probably often confounded with other skin diseases. Nevertheless there is no doubt that true leprosy has existed from time immemorial. Ancient prescriptions for treatment have been found in Egypt and given a probable date of 4600 B.C. Its origin is supposed to have been along the banks of the Nile, but the early

history of nearly all countries gives proof of its widespread occurrence. As early as 1500 B.C. in India and in 600 B.C. in Persia, history shows measures were taken to control it. Aristotle and later Greek writers describe the disease. Hippocrates does not mention it, though the name "leprosy" derives its name from his "lepra" or "scaley" disease, which was no doubt psoriasis.

During the middle ages leprosy became widely diffused in Europe. The Crusaders probably brought it back with them and helped in spreading the disease. It became necessary to segregate the afflicted persons, and every considerable town had its "leper house." Italy had established lazarettos or pest houses as early as 636 A.D. About the fourteenth century the total number of such houses in England, France, Spain and Germany has been reckoned at 19,000. The earliest one in England was established at Canterbury in 1096, and the last one at Highgate in 1472. At one time Great Britain alone had 95 religious hospitals for care and treatment of lepers.

During the fifteenth century the disease underwent a remarkable diminution. It practically disappeared from civilized Europe and the leper houses were given up. The general disappearance is the more unintelligible because it did not disappear everywhere. It lingered in Scotland until the nineteenth century. At the present time it still exists in Norway, Iceland, along the shores of the Baltic, South Russia, Greece, Turkey, Spain and Portugal. Teutonic races seem to be especially free from the affliction. The disease did seem to be dying out except in Spain where some increase had been noted—but now the spread of leprosy in France has recently been called to the attention of the French Academy of Medicine.

An item in *Science* which appeared early in 1926 states: "A report by Dr. Janselme shows that this terrible disease, usually associated in the public mind with far-off corners of the earth or with times gone by, is threatening modern France. In Paris alone there are at the moment nearly 200 cases of leprosy. These cases may be divided into two classes—the wealthy, who can take care of themselves and present little danger of infecting the public, and the poor, who do not go to a doctor and do not find out what is the matter with them until the disease has entered a serious stage. As a result of this unexpected communication, the Academy of Medicine resolved to make representations to the government. It was recommended to the government that strict surveillance should be exercised over all lepers, that they should be excluded from schools and from all professions bringing them into contact with the public, that poor lepers should be placed in hospitals or sanitariums and maintained there at public expense, and that a clean bill of health should be demanded from all immigrants or travellers coming from countries where leprosy is prevalent."

The prevalence of leprosy is extensive throughout Asia, from the Mediterranean to Japan, and nearly all parts of Africa. The Chinese introduced it into Australia and the Hawaiian Islands, where it has increased rapidly. Within our own boundaries we find it among the Chinese in Louisiana and California, and the Norwegians of the Northwest.

The scientific development of the treatment of leprosy has been carried on almost exclusively in Hawaii, the Philippines and Calcutta. Since Hawaii and the Philippines belong to the United States, a few words might

be said concerning the present status of leprosy in these islands. Not until 1848 was there any leprosy in Hawaii, when it was found among Chinese laborers. The number of cases increased very rapidly until in 1882, 4,000 cases were reported. It is probable that at the present time the numbers are somewhat smaller, but leprosy is still a very prevalent disease. A colony of lepers exists at Kalihi in Honolulu, where a few hundred cases are being treated continuously and a scientific study is being made in cooperation with the University of Hawaii.

In the Philippines it is estimated there are about 16,000 cases at the present time, of which number probably 5,000 are segregated. The large, well known colony is at Culion. This is established in such a way that many of the leper patients can take an active part in the administration, more than half of those employed in administrative positions are lepers, as are the teachers. In addition there is a large staff of non-leper physicians, nurses, etc. Not so very long ago the late General Wood made a public statement requesting that citizens in this country contribute to the fund for caring for the lepers. It is still one of the most serious problems facing the Philippine government. This can be seen when it is considered that $600,000 a year, two per cent of the entire revenue of the Islands and one-third of the health service appropriation, is given over to the treatment of leprosy. The caring for lepers is particularly difficult because the patients are generally not found until the disease is in an advanced stage.

Transmission and Pathology.—The disease is due to the Bacillus Leprae, which was isolated in 1874 by Hansen. The mode of transmission of the disease is not well understood. It is certainly contagious, as it is always

found in people brought socially together. It is not hereditary, as has been shown conclusively by recent work at Culion, where new-born babies, if taken immediately away from leper-mothers, do not contract leprosy.

The disease causes a great multiplication of cells in the affected tissues which thicken and degenerate. The bacilli are found in these cells.

The disease is now known in three types—the so-called nodular, the smooth or anesthetic type, and the mixed. In the nodular there is noticeable in the patient at the beginning, irregular fever, vertigo, no appetite and other similar symptoms. Dark red, coppery patches appear on the face, hands, and feet, about the size of a quarter. Gradually the skin thickens and nodules appear which are pink at first and then brown. The face is generally drawn and the afflicted persons frequently take on the appearance of a lion. The tissues of the eyes are affected next. The mucous membranes of the nose and throat are impaired so that the breathing is difficult and a raucous voice is produced. The nodules then break down, ulcerate and leave sores. The patient becomes weaker and dies of exhaustion or generally of some intercurrent disease such as nephritis, cerebral degeneration or tuberculosis. The disease, after it once starts, generally extends over a period of two years.

The smooth type is less severe but more chronic. The eruption consists of patches of dry discolored skin not elevated above the surface. The cutaneous nerves are affected and small blisters appear. The patches enlarge, sensation is lost and muscular power decreases. Along with these symptoms is found contraction of the tendons, the nails become hard and clawed and finally

perforating ulcers cause the ears, toes, or fingers to die and drop off. Finally general paralysis occurs. The disease may extend over a period of twenty years if the patient is cared for, and sometimes recoveries without any special treatment are reported. In general the disease as it is now found combines the nodular and the smooth type.

Treatment.—The treatment of the disease until very recently has been empirical and is largely so even now. In general the patients are given a full diet, care is taken that they receive digestible foods, hot baths are administered, and particular care is taken against complicating diseases such as malaria, pleurisy, and pneumonia. The patients are almost always given a tonic and in addition receive a chaulmoogra oil treatment. It has been definitely established, however, that non-medicinal treatments have been found utterly inadequate in the absence of medicinal treatment. The chaulmoogra oil treatment is the only one known which has been found to be of distinct aid to patients with the disease. A considerable portion of those patients who are discovered within the first six months after infection can be cured by the general treatment just mentioned. The older cases are relieved to be sure, but the percentage of cures is small.

Chaulmoogra Oil.—The use of chaulmoogra oil and other oils of similar character has developed from the observations noted during the past several centuries, that natives in the Far East afflicted with leprosy knew that they received relief by rubbing the wounds with the oil and by oral administration. The date of the discovery of this oil and its efficacious properties is unknown. It is like so many of our important drugs at the present time. They have come down to us from uncivilized

peoples, and science has found nothing which can surpass them. Quinine, morphine, and strychnine may be mentioned particularly.

The chaulmoogra oil used in the treatment of leprosy has until very recently almost always been secured from the fruit of the Taraktogenus Kurzii. It resembles the grapefruit in appearance and when cut open is found to contain irregular shaped seeds. These seeds are pressed and the crude oil which is a thick semi-solid, yellowish brown mass at room temperature, is obtained. Other oils of importance are those of the Hydnocarpus Wightiana and Hydnocarpus Anthelmintica. With the increased interest other sources have been sought and many species of plants have been discovered during the past fifteen years which contain oil of a similar chemical character and usefulness.

In the olden times the first treatment was percutaneous along with internal administration. The oil, however, causes rather violent intestinal disturbances with nausea and sickness. It was a superstition among the natives that if a patient could take the oil without becoming sick he would get well. This is not true, though many of the patients were materially improved.

Chemical Improvement of Chaulmoogra Oil.—It was not until 1899 that any attempt was made to study chaulmoogra oil from a medical or chemical standpoint. At that time an attempt was made to inject this oil subcutaneously. A marked improvement in the patient was generally noticed, but the injection proved to be very painful. This was generally assigned to the fact that the oil being so thick was slowly absorbed. As a result of this early observation many other physicians offered preparations which were less viscous. Thus one

of the first suggestions was the admixture of chaulmoogra oil with olive oil. This was followed by a number of others of which that proposed by Heiser gave the most success (camphorated oil, chaulmoogra oil, resorcinol). From the years 1899 to 1918 this was the favored remedy. Just recently, however, it has been definitely established that refined chaulmoogra oil, or especially the oil heated with one-half per cent iodine, is superior to any of the admixtures previously prepared, and a minimum of irritation occurs. Still more recently Dr. Johansen of the United States Public Health Service reports that the injection of chaulmoogra oil containing benzocaine, a local anesthetic, relieves the patient of much pain and therefore makes it possible to give the leper a definite amount of oil over a definite period of time.

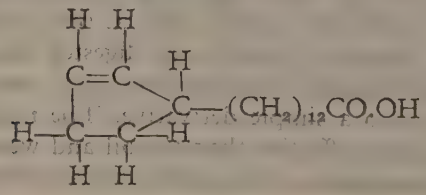
In 1918 Dr. Rogers, a prominent British surgeon in India, converted chaulmoogra oil by means of caustic soda into the corresponding soap just as palm or olive oils are converted into ordinary soap. The soap (sodium salts) was dissolved in water and the solution injected, first subcutaneously, then intramuscularly and finally intravenously. This treatment proved more satisfactory than any that had hitherto been given and is still used to a certain extent in India and China in the leper colonies. About the same time Dean, president of Hawaii University until very recently, but at that time a member of the chemistry department, digested chaulmoogra oil with alcohol and a little sulfuric acid, thus converting the oil into a simple derivative. The product thus obtained is more effective than the oil and was administered intramuscularly with much success. Many of the

lepers in the Philippines and Hawaii are treated by this procedure.

Rogers at the same time showed that certain entirely different oils might be used. In particular cod liver oil, which had no effect by mouth, was of distinct value when injected intramuscularly or subcutaneously, either in the form of the oil itself or its simple derivatives. In general, the results were uncertain and not so satisfactory as when chaulmoogra oil or derivatives were used.

The result of these medical and chemical studies is that for every leper effectively treated eight years ago, probably 100 are effectively treated today. Moreover, the scientific worker has now a solid foundation of knowledge about lepers upon which to base future work.

Future Progress.—But the advance in the treatment of lepers is just beginning. Undoubtedly further improvements may be expected in the mode of treatment with chaulmoogra and natural oils or their derivatives, but the hope of the leper is much more likely to lie in the discovery of synthetic products which are more efficacious than the natural, more standard, and perhaps less toxic. The organic chemist has studied that portion of chaulmoogra oil which has the curative effect, the chaulmoogric acid molecule, in such great detail that the internal nature or structure of the molecule is now understood. It has the formula represented by:



The preliminary study of chaulmoogric acid and its derivatives indicated the probability that the ring structure was one of the most important parts of the molecule causing the bactericidal effect. This ring has been simulated in synthetic molecules and the new compounds thus produced have been shown to be bactericidal toward B. Leprae *in vitro*. In fact the —COOH group can be in a different position in the molecule than at the end of the chain as in the natural product and the synthetic substance thus obtained is more bactericidal than the natural. Already many compounds containing various ring structures have been prepared, the first few of which were rather difficult to obtain. On further study simpler compounds of bactericidal character have been discovered, and still further improvements may be expected in the future. The general results have thus far led to the conclusion that perhaps the physical properties of the acids as produced by the particular structures of the molecules, are a very important contributing factor to the bactericidal action.

The task of creating by synthesis a compound of sufficiently high activity against the Bacillus Leprae, with sufficiently effective penetration into the lesion and sufficiently low toxicity to the patient as to constitute a marked advance beyond the present known remedies, does not appear to be an impossible one. Even though the particular leads which have been started should fail, there is no question but that sooner or later scientific study will succeed in finding a remedy which, even if it does not entirely eradicate the disease, will at least relegate most of its horrors to the past. The most important factor for the future is treatment of leprosy as a preventive measure. In the past, segregation in one form

or another was the only available preventive measure, but in many countries effective segregation is too enormous a task to be considered. In India, treatment is already the most important item on the eradication program and it is the only reasonable hope for the control of leprosy as a world problem.

Chapter IX—Article 7

THE WAR ON THE HOOKWORM*

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Colonel Ashford has been an army medical officer since 1897 and has just been retired from active service. Since 1926 he has also been Professor of Tropical Medicine and Mycology of Columbia University, with activities in Porto Rico in the School of Tropical Medicine. Colonel Ashford was in charge of the pioneer work in the war on the hookworm carried on by the Porto Rico Anemia Commission in 1904; he was a member of the medical expedition to Brazil organized in 1916 by the Rockefeller Foundation and has rendered other outstanding service to the world in the field of tropical medicine. He was a U. S. delegate at the International Congress of Industrial and Alimentary Hygiene in 1911. Colonel Ashford received the Distinguished Service Medal for his services during the war, in which he had charge of the battle training of medical officers, and has been the recipient of many other honors abroad as well as in this country in recognition of his distinguished services.

Hookworm disease, or uncinariasis, is one of the most widespread of diseases in large sections of the world. It saps the vitality of millions of sufferers, it lowers their resistance to other infections, it destroys their economic power to earn a decent livelihood. Chemistry has placed at the disposal of medicine remarkably successful weapons to combat this disease, weapons which are also noteworthy because of their simplicity and availability. However, a successful war on the hookworm involves important factors other than treatment with these simple chemical remedies. Because the disease affects large proportions of whole populations of countries, in localities where ignorance and poverty are likely to predominate, a wisely directed strategic campaign is necessary in which

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education and stimulation to certain social reforms are as vital as effective medical treatment. Moreover, the campaign has had to elaborate measures for treatment, not of individual sporadic cases but of whole communities, at a cost that would not be prohibitive. In a word, the war on the hookworm presents a picture of splendid cooperative effort, which is not only conquering one of the most common diseases of mankind, but which in many respects is also a model for widespread campaigns of the future against other common diseases. In this campaign, the weapon that actually dislodges the enemy is provided by chemistry. That is the picture our story will unfold.

The Disease.—Uncinariasis is a specific communicable disease, common to most tropical and subtropical climates, and endemic in favorable localities, such as mines, tunnels, and brickyards in temperate zones. Indeed, some of the most heavily afflicted countries in the temperate zones are breeding grounds only in the warm months of the year, and are completely sterilized of their larvae in the winter, only to have their soil reinfested from the human reservoir of the worm when summer returns. Uncinariasis occurs in individuals who come into intimate contact with damp earth or muddy water containing the larvae of Ancylostoma duodenale or Necator americanus, the two species of hookworm which cause the disease. It is characterized by an insidious, progressive anemia, debility, and various digestive, circulatory, and nervous disturbances in cases sufficiently infested to produce a definite clinical picture; by underdevelopment of children, reduced resistance to other diseases, lack of mental alertness and physical vigor, with consequent loss of laboring efficiency in whole communities in which by

lighter infestation a depressed sociologic state is induced. It is capable of cure on expulsion of the parasites brought about with the aid of certain chemicals, and of prevention by a proper disposal of human excrement.

The adult worm is a little over half an inch in length, of the thickness of a hair, and the sexes are differentiated. The name "hookworm" was given the worm of the genus *Uncinaria* infesting the fox, on account of its possessing hooks in its tail expansion. The allied worms infesting man thus received a similar sobriquet. The female produces around 9,000 eggs a day, microscopic in size, and incapable of developing into larvae until they reach the soil where under propitious circumstances they attain the stage of infective larvae in about 120 hours. The size of these larvae just permits of their being seen with a small pocket lens.

These tiny larvae in contact with the skin of man, generally the bare foot introduced accidentally into a nest, penetrate the skin and reach the circulatory apparatus. They are caught in the capillaries of the lung, rise through the bronchial tubes to the trachea, and are swallowed, thus reaching their preferred feeding ground in the small intestines. Here they bury their heads in the mucous membrane and begin their sexual life.

Thus, in contradistinction to infection, a man with one hundred worms will never have another until he acquires it through the skin from the outside world, generally as above described.

While the mode of production of the anemia is still a mooted point, and, in reality, is due to a combination of factors, "bloodsucking" is not the principal one. The theory sounds well but it is not borne out by the facts. The emission by the worm of a specific toxin is a reason-

able supposition but many of the symptoms believed to be peculiar to the disease are undoubtedly exaggerations of nutritional deficiencies seen in other tropical affections.

The clinical picture of a light case is that of a previously vigorous individual who for no apparent reason becomes a mild dyspeptic and gradually loses in color, strength, and initiative. He is considered ailing by his friends and often lazy by less indulgent acquaintances.

In moderate cases, "indigestions" are more prominent, anemia becomes definite, circulatory disturbances with palpitation of the heart and breathlessness on exertion, giddiness, and buzzing in the ears are noted, and a demonstrable reduction in efficiency for labor is manifest. Above all is noted the dullness of intellect; the victim is depressed, languid, confused or seemingly stupid.

In severe cases, all of the above symptoms are intensified, especially the anemia, and serious circulatory impairment takes place with great breathlessness on exertion, extreme weakness, and a tendency to edema. Such a person, if not bedridden, at best can work but a day or two each week and becomes a charge upon his family, his employer, or the State.

The hookworm state differs from clinical uncinariasis in that the effects of infestation are chiefly seen in the development and mental condition. Children are stunted in growth, their complexion is sallow, their hair is brittle, their skin wrinkled and dry. The bony structure is underdeveloped, particularly that of the chest, and the subject is underweight. Infantilism is common among adolescents, not only a retardation of body but of mind and character, and resistance to other diseases is much lowered.

The Problem in Porto Rico.—In the entire history of uncinariasis there has probably never been a country in which such an appalling number of deaths from uncomplicated uncinariasis have occurred as was witnessed in Porto Rico just before and after the occupation of the Island by our forces.

The pioneer work of the Porto Rico Anemia Commission of 1904, the first campaign in the world against hookworm disease as a scourge of agricultural laborers, was based on treatment of all infested.

The problem which presented itself to this Commission was that furnished by a rural agricultural class of some 800,000, ninety per cent of whom were heavily infested with hookworms. The total death rate from all causes on the Island, whose population was 943,000, lay between 35 and 42 per thousand, one-third of which was attributed to "anemia." A preliminary survey of 549 persons of this agricultural class gave an average hemoglobin percentage of 43, and wages faithfully portrayed their inefficiency: from ten to thirty cents a day in the coffee plantations, around seventy-five cents in the coastal sugar lands.

The two main points of attack were, first, ignorance of the nature of their disease; second, the disease itself, easily curable at that time by the chemical, thymol. The obstacles to a successful attack were unique. The education of the ruling classes was more difficult than that of the worm-sick. Even had we succeeded in having a latrine law passed, we would have only secured the ill-will or contempt of the very people we desired to reach. The idea was firmly fixed in the minds of rich and poor that "anemia" was due to miserable food, exposure to the elements, and a tropical climate. The preaching of plati-

tudes on correct hygiene, the asseveration of what to them appeared an outrageous notion of cloistered Science, and the experiment of building latrines and buying shoes, to be exacted by law at the cost of unfortunates who could barely get enough to eat, and not enough to eat normal food, would have defeated our war on the hookworm in those early days before a blow against the disease could have been delivered. Sanitation can be the main issue today; it can, and rationally should precede all future treatment campaigns, but this is due to the world-wide knowledge among at least an enlightened majority, and always among the ruling classes, that it is rational and just.

But in those days no such background was afforded. The educated, cultured and polite gentleman of the old school, plantation owner or legislator, was even more ignorant of the nature of uncinariasis than the poor. unlettered sufferer from its effects. We decided to first attack the disease, and let our personally instructed and cured patients talk for us.

Five years had gone by since the hookworm had been first identified as a rural scourge of tropical and subtropical America, a find made in Ponce, Porto Rico, by the writer on the 24th of November, 1899. It was seen in studies made during those five years that intensity of the disease depended on the number of worms harbored. In selecting the type of campaign to be followed it was instantly recognized that the worst cases would be naturally the first to solicit our services, and that in addition to the tremendous force of an ocular demonstration that anemia in Porto Rico was not due to poor food and climate, but to a worm that could be expelled by a few doses of thymol, we would infallibly reduce the infesta-

tion of the soil. Thus, if we should manage to cover with our activities the more heavily infested mountainous interior, and could reach by treatment only one-half of the infested population, we would get most of the worm-sick and reduce soil infestation by over two-thirds.

All of these things are clearly expressed in the first report of the Anemia Commission of Porto Rico of 1904, and repeated and added to in "Uncinariasis in Porto Rico," Senate Document 808, 1911.

Chemical Warfare on the Hookworm.—For the above-stated reasons, the very positive and practical chemical solution of the problem was selected. It was to be war, not a series of parleys and conferences. Our standard weapon was thymol and we carried the latrine idea, the shoe idea, and the humanitarian idea along with us to weaken the enemy. Had we not done this we would have failed. We felt justified in leaving the devastated areas to be built up in the coming times of piping peace.

In seven years of an unremitting struggle, 317,000 of the estimated 600,000 inhabitants of the hills were treated. The mortality in Porto Rico which had already begun to fall without any general campaign of treatment, now definitely fell to half of what it had been in 1900. The laborer's efficiency, estimated by a rise of nearly 30% in hemoglobin and by the testimony of 224 of the principal planters of the Island, rose 67%, and the people of Porto Rico were at last thoroughly convinced, chiefly by the "jibaro" or laborer himself, that "anemia" in this land was generally due to a parasitic worm planted through ignorant carelessness in the soil.

The war was definitely over. The machine was turned over to function under the Health Department of the

Island in 1911 as a routine measure in holding down morbidity and mortality in the Island of Porto Rico.

Work of the Rockefeller Foundation.—In 1909, just as the last battles against uncinariasis were being waged in Porto Rico, was born the Rockefeller Sanitary Commission, later to become the International Health Board of the Rockefeller Foundation. At first the ruling instinct in this splendid body of men was to save our own people of the Southern States, shown by Charles Wardell Stiles to be also in the throes of uncinariasis. But in this case the battleground was chosen. In the United States habits of thought as to cause and effect were somewhat different. Latrines and shoes were made the crux of the campaign; treatment was at first less stressed, perhaps. Above all, cooperation with existing health organizations was to the fore. And herein lay the marvelous success of what is now the outstanding agency for the application of the medical sciences in the prevention of disease the world over.

It was least of all money, which fortunately has never been lacking, owing to the great philanthropist whose soul conceived this boon to humanity, but methodical administration, brains, diplomacy, and honesty of purpose that has made the Rockefeller Foundation an international household word.

But this time there was a background. The campaign and its results in Porto Rico were by no means unknown in the Southern States, and this knowledge had its effect on the reading public and the governing classes.

The plan of campaign of the Rockefeller Foundation has always rested on three great principles:

1. A preliminary survey of the field of proposed operations.

2. A preliminary sanitary campaign in cooperation with existing health departments or recognized agencies.
3. More or less intensive treatment of given areas as a proof to the public of the benefits to be derived from expulsion of the worm, accompanied by an incessant campaign of propaganda and instruction in the principles involved in the prevention of infestation.

The relative number of infested in a locality or a whole country, expressed in percentage, was the guide for many years in determining the seriousness of a hookworm problem. Up to December 31, 1915, 1,217,483 persons had received a microscopic examination of their feces in ten Southern States and 32.6% were found infested.

Even as early as 1915 the Foundation had carried its work into British Guiana, Dutch Guiana, Grenada, Santa Lucia, Saint Vincent, Trinidad, Costa Rica, Guatemala, Nicaragua, Panama, and Egypt, examining from 2,380 to 62,391 persons in each country and finding from 43.9% to 83.6% infested. The average infection rate for these tropical countries was 60%.

In 1917 the statement was made that the hookworm belt lies between parallels 36 N. and 30 S. and that it contains more than half of the world's population. In 1919 the work was continued in all countries in which it had begun and extended to include Ceylon, parts of China, the Seychelles Islands, Siam, Queensland (only province in Australia which had a hookworm problem), and Brazil, where in 1916 the writer, at the Foundation's invitation, made the first survey with Dr. John Ferrell and Dr. Richard Pearce.

The survey made in Colombia developed the fact that while the hot tropical lowlands had an infection rate of 84.1%, as ascent of the Andes was made, this rate gradually fell, until in the higher altitudes it disappeared. In other words, countries with a night temperature of 50° F., or below, for a considerable part of the year have no serious hookworm problem.

In this year was developed in China the worm-index as a measure of severity of infection, the Commission in the Orient reporting that for every 12 hookworms harbored, a fall of 1% of hemoglobin might be expected. This cast the first shadow over the validity of the infection rate as a guide to the amount of infestation and the consequent seriousness of the problem for an infested country.

The question of reinfestation is thus discussed: "Even though in certain of the areas mentioned from 50% to 100% of the persons formerly cured of the disease have since become reinfected, there can be little doubt that as a result of treatment the average number of worms harbored by each infested person has been very materially reduced." The problem of hookworm control is seen to be one of mass infestation and previous treatment certainly reduces this. In this report it is also pointed out that in Brazil worms are slowly acquired throughout life. Children who begin to work in the fields at eight years of age start with an initial infection of 15 worms and are apt to acquire about 50 worms a year. Those who do not work in the fields acquire about eight a year. In families using shoes only an average of 40 worms were expelled; in those without shoes 226.

In 1921 the reinfestation problem again claims attention. In Brazil it was found that an enormous reduction

in numbers of worms harbored had been secured by chemical treatment although the infection rate remained practically the same due to weak reinfestations. In communities where lively latrine building accompanied treatment these infestations were even lower.

In 1922 the Rockefeller Foundation reached a conclusion which simplified the problem considerably. Heretofore, effort had been directed toward expelling all hookworms, and not only the Foundation but the Porto Rico Anemia Commission had found the remaining 5% of worms more expensive to eliminate than the first 95%. It was also not without increased risk that these vermifuges were constantly repeated, and, worst of all, it caused a falling off from attendance due to the constant drugging of the people who came to our dispensaries. In the vast majority of cases, the disease disappears after two or three administrations of a good anthelmintic, such as thymol.

In this report we find the Foundation speaking of "mass treatment," meaning treatment *en masse* without previous microscopical examination, of a whole community whose infection rate is high and who harbor an average number of worms sufficient to produce a deteriorating effect on health or constitute a menace to others. Smillie, in Brazil, found no clinical symptoms in patients harboring less than 40 worms. Darling, in the Orient, makes this number 50. This distinction between worm-sick and worm-carriers had for a long time been expressed by the Germans and by ourselves.

In 1923, an advance in the value of a hookworm survey was made which has literally revolutionized the workings of the Foundation: the estimation of the number of worms harbored from the number of eggs in

a specimen by Stoll's method. Thus, by combining the information yielded by Baermann's larva index in soil and Stoll's egg-count to arrive at the worm-index in the patient, the Foundation is rightly jubilant over the prospect for the future: "In the past, resurveys have failed to show the full accomplishment of the original campaign because of the absence of quantitative information. This need no longer be true."

This statement should modify once and for all the objection that treatment of hookworm brings only an ephemeral betterment of the hookworm state of the community and that rapid reinfestation soon reproduces the same lamentable picture which originally provoked action against the disease.

Treatment alone is certainly inferior, quite inferior, to treatment plus education in the proper disposal of human excrement and enforcement of privy laws, but that the effect of general treatment is lost in a few months or years is shown to be an error by Cort and Hill, representing the Rockefeller Foundation, who found in areas worked over twenty years before by the Porto Rico Anemia Commission only about one-seventh of the degree of infestation demonstrated in areas untouched by this Commission in the Island.

This is in accord with the popular evaluation of the campaigns of the old Porto Rico Anemia Commission. It is deep in the conscience of the people of Porto Rico that the intensity of the almost universal severe anemia of the peasant is but a shadow of what it used to be, largely as the result of those early labors. These early campaigns perforce were mainly waged with chemical weapons. The reason therefor has been explained.

In 1925 the fallacious "infection rate" retires well to

the background and the true and precise appreciation of the degree of hookworm infestation shines out from every corner of the world to which the Foundation was carrying its marvelous message to health. From China, where from time immemorial the scientific world had imagined heavy infestations from lack of latrines and the utilization of human dung to fertilize soil for garden products, came startling news: From Cort and his associates, that group who have done more than any other body of investigators to make a judicious solution of this world problem possible, we learn in a report of operations conducted in 1923-24 that in North China very little hookworm was to be found and that in Central and Tropical China only an irregular distribution, due, first, to the death rate of ova and larvae by the storing of human excrement previous to its use as fertilizer; second, to its drying before use; third, to its submergence in the water-flooded rice paddies, also fatal to this period of hookworm development. On the other hand, wherever the employment of raw feces for fertilizer was a practice, wherever large bodies of laborers worked over soil-polluted earth in propitious regions, the same intensity of infestation with identical results, as in all other countries, were reproduced.

The work of the Foundation was now re-oriented. A plan of combat was sketched in which those suffering from the disease, uncinariasis, and not those merely harboring a few worms without detriment to their health, should receive preferent attention. This was combined with an intense campaign for the proper disposition of feces and education in prevention of infestation. This plan was believed to promise a quicker remedy for acute situations in countries as yet untouched, enormously

reduced cost of operations, suddenly broadened the sphere of the Foundation's influence, and lessened tragic intoxications which, although extremely rare, reacted strongly on the community in which they occurred.

In Tennessee the bulk of the campaign fell among the barefooted rural school children who were the greatest sufferers, and all regions yielding over 25 worms per head were given mass treatment for the children. In this State it was also noticed, from egg-counts and counts of larvae in soil, that the average number of worms harbored in certain regions was dependent upon the unsuitableness of the soil for the development of infective larvae.

In 1926, the last published report of the Foundation to date, the conclusion is reached that all countries with thirty inches of rain a year and an altitude of less than 5,000 feet, with shade, soil, and temperature propitious, were almost certainly exposed to more or less grave infestations. Factors of great importance were also agricultural occupation and poverty which prevented the buying of shoes and building of latrines. It appears that droughts, heavy rains, and sharp declivities make for light soil infestations.

The most interesting news comes from India. Here the contemplation of an infection rate of 90% (Madras) in the heart of a country with a population of 300,000,000 had previously left the Foundation appalled at the magnitude of their problem. Now, however, by means of egg-counts, 13,924 persons were found to yield only 873 ova per gram, or 40 worms per person. In another part of India it was two or three times greater but the indefiniteness and hopelessness of the situation have disappeared. With Stoll's scouting plane all of the enemy's secrets are known.

In all, the Foundation had examined from 1910 to December 31, 1925, 5,161,668 persons, and found 3,053,442 infested, an infection rate of 59.2%.

TREATMENT

We shall now see that every dose of a specific vermifuge is an infantryman in this war. The nascent Foundation, in the person of its first director-general, Dr. Wickliffe Rose, had an opportunity to visualize this in 1908 while on a visit to Porto Rico, made to look over the operations of the Anemia Commission of that island. The working basis of this Commission was the rural dispensary. This was also the plan first adopted by the Rockefeller Sanitary Commission, the predecessor of the Foundation, but it contained modifications adapted to the people of the Southern States. It has been referred to by the Foundation as the "dispensary plan."

Very soon, however, the itching from which we ourselves had ardently suffered, to *"de-hookwormize"* a whole community and thus chemically disinfest man, woman, and child, took form in what the Foundation denominated the "intensive plan." It only took a short time to convince them that this plan was not as feasible as it sounded. In three years these communities would too frequently return by reinfestations to an uncomfortable infection rate, a discouraging phenomenon which at that time the Foundation was not in a position to understand.

From 1910 to 1915, by the dispensary plan, practically the whole infested area of eleven Southern States was covered, the press, the schools and the practicing physicians were all educated as to the nature of uncinariasis, about 750,000 persons were treated, and about 20,000,000 recognized the importance of the disease and

its prevention. An impressive by-product of latrine building was the immediate falling off in the prevalence of dysentery and, especially, typhoid fever. As a rural and even urban disease typhoid rapidly fell away in Virginia from 14,400 cases in 1909 to 5,038 in 1917, a reduction of 65%. On the other hand, the mortality in influenza was found to be twice as high in the hookworm infested as in the non-infested areas.

Such campaigns, as especially seen in Porto Rico, raised the birthrate and increased the educational efficiency of schools.

In 1919 we begin to hear of mass treatment, already defined and really a modification of the intensive plan but shorn of its disqualifying features. The proposition was tentatively made to give two treatments of chenopodium to all save the manifestly worm-sick, who should receive three, in countries known to be heavily infested, this without previous microscopic examination. It is shown in 1920 that inasmuch as 95% of worms are expelled by two treatments and 99% by three, and as there is a possible 50% failure of microscopical diagnosis in the lightly infested, it would be good policy and an economy of time and money in heavily infested localities to treat without microscopical examination all save 10% to 20%. The latter should be examined before treatment to act as a check upon the unexamined. The only persons to be eliminated from such treatment would be women pregnant three months, children under a year, and those refusing treatment or physically unfit to take it. Ten days after the first or second treatment all should be examined.

Smillie's plan of mass treatment for Brazil seems peculiarly felicitous:

For lightly infested areas: All cases positive by micro-

scopic examination are given two treatments, save field laborers who receive three.

For heavily infested localities: All persons who spend from ten to twelve hours in the fields daily are given a preliminary course of three treatments. A simultaneous effort is made to construct latrines in at least 75% of houses. In the final course only those positive by microscopic examination are treated and these receive one treatment only. A permanent staff is left thereafter to work in the area.

For communities that cannot or will not build latrines: Two treatments will expel practically all worms. No large complement of fresh worms to replace those expelled will bring about appreciable symptoms until at least three years have passed. This is avoided by treating all in the community once a year.

This new point of view begins to weaken the dispensary plan, the objections to which include a long wait to see the doctor, as well as a weary trudge to get to him, for in applying mass treatment the remedy can be carried to the home.

DRUGS USED

Thymol.—Up to 1915 thymol was practically the only weapon used. It was very rarely toxic, expensive, caused burning in the stomach and a disagreeable aftertaste, and only expelled 95% of worms after three doses, a week apart.

Chenopodium.—In 1915 chenopodium was shown in Java to have a remarkable effect in expelling hookworms and with them the common round worm, Ascaris lumbricoides. It began rapidly to displace thymol, especially in the Orient. Of 13,000 cases treated

by it, 73% were cured in two administrations; but it is at times toxic, generally in children under twelve, and for this reason Darling reduced the dose one-half.

The advantages of chenopodium over thymol were soon apparent. Where 23.6% of failures to remove all worms were registered by thymol, only 7.6% of failures occurred when chenopodium was the anthelmintic. Chenopodium is also more potent against Ancylostoma duodenale and is far less unpleasant to take. Its cost is 2.76 cents per individual as against 9 cents for thymol.

Carbon Tetrachloride.—In 1921 Maurice Hall discovered the extraordinary power of carbon tetrachloride in expelling hookworms in dogs without producing serious intoxication, and in 1922 we see it heralded as the most energetic anthelmintic for the expulsion of human hookworm. It has been found especially felicitous in combination with oil of chenopodium in a mixture of two parts of carbon tetrachloride to one of the oil of chenopodium: 2.4 cc. of this mixture is a dose for an adult male and one-eighteenth of this dose per year of age is suitable for a child. Carbon tetrachloride has a selective action on the females of Necator americanus but is toxic in some rare cases, particularly when impure, and is cumulative in its effects.

In preparation for the dose, diet restrictions are not so important and food may be taken one or two hours after its administration. It has a selective toxic action on the liver but this seems a rather remote danger, for even in cases so affected the organ rapidly recovers. Among the advantages of carbon tetrachloride are its palatability, its great efficiency, and its inferiority in toxic action as compared with chenopodium. The exception is found is alcoholics, who bear it badly. It is

much easier to administer than thymol or chenopodium and may be safely given to children and pregnant women. From a personal conversation with Dr. Frederick Russell, Director General of the International Health Board, the writer understands that evidence tends to show that the poisonous effects of carbon tetrachloride can be successfully treated and even forestalled by treating a previous blood-serum calcium deficiency. It has been found that the poisonous effects of the drug are best avoided by good and prompt purging after the administration of the anthelmintic.*

In the total work of the Foundation up to December 31, 1925, 5,110,359 persons have received one treatment of some one of the specific anthelmintics, and 2,687,640 two or more treatments.

OTHER MEASURES

Latrines.—It requires no literary ability to demonstrate that the real, the permanent solution of hookworm disease problems is prevention of soil pollution. That even such an impolite thing as a latrine can become an ideal in an effort to elevate mankind toward physical perfection is also apparent. But while the sanitary latrine is a practical object to be exacted by law from literate American agricultural laborers, it is still a remote sanitary idea for those unschooled hordes, sunk in abject poverty, who read their lessons in Nature.

The crusade for latrine construction is an altruistic and scientific one, an international one, which recognizes but one Fatherland, the common one of all

*An article by Lamson, Minot and Robbins in the *Journal of the American Medical Association*, February 4, 1928, gives full details as to the causes of carbon tetrachloride intoxication and the steps to be taken to prevent the same.

humanity. But the world has not yet been consolidated under one flag, and as long as political divisions of the earth exist, that most intimate feature of family life, the cleaning up of one's own house, cannot be done by outsiders. For this reason, with excellent judgment, somewhat tinged by necessity, the Foundation decided from the first to energize latrine building by working upon the existing health organizations, local and national, and let them exact it, for a thing is unappreciated unless we take some part in earning it for ourselves. Indeed, the Foundation has even gone further and turned the whole campaign over to such local organizations as give definite indication of enthusiastic and self-sacrificing interest in the spirit of this war. The stern fact remains: we must avoid soil pollution. We must have latrines. It is the least a great philanthropic organization can demand of us before they will consent to work for us. Sanitary law is more than justified in exacting them of a poverty-stricken people, for much of their poverty too often depends on uncinariasis.

Propaganda; Schools.—In no locality in which it has worked has the Rockefeller Foundation neglected a single opportunity to acquaint the people with knowledge of their little enemy. At dispensaries, in pastoral visits of their inspectors to homes, in theaters, churches, in every sort of gathering of people together for whatsoever might have been the object, this gospel of health has been steadily preached. Of all means of reaching the people, the cinematograph is easily the winner. Thousands upon thousands of lectures, demonstrations of worms, of privy building have been made the world over, and their strongest ally has been a patriotic press.

Some of the methods of propaganda are among the most ingenious known to Medicine—even to Patent Medicine! The astute Payne, one of the unforgettable group of investigators who put the scientific organization of the modern hookworm campaign on its feet, told the writer that in some oriental country or other he found it necessary to adopt the mystic story-telling of the Oriental races to "put over" his message. Like the Great God Thing-a-bob he sat and charmed his hearers with tales of the great god something-or-other whose well-beloved people had become cursed by some divine friend of his, who had sent a scourge of body serpents to invade his people. These serpents were sown in the soil by the following of the divine traitor and sprang up to attack the great god's good and well-beloved people as they innocently tread upon the earth in pursuit of health, happiness and lucre. One day, however, word was passed about that every follower of the gracious god was to collect his excrement in a pit and smother the sons, and daughters, of these vile serpents, and from that time the people were freed of their sufferings, made untold riches and lived happily ever afterward.

All of this was copiously illustrated in colors by a native artist and exhibited; and, like Susan Smith's Soothing Syrup, the idea was sold to the populace, who meekly swallowed the worm-killer next day and hastened home to build themselves a temple to health.

But of all propaganda, especially for the inevitable village wise-guy, there is one form of advertising that never fails: the cure of the sick, under one's very nose. So, after all, chemistry again is back of words, back of

the wonderful pictures that man's spiritual self paints to make them real.

SCIENTIFIC RESEARCH ON THE CONTROL OF HOOKWORM DISEASE

It is certainly not claiming too much to say that fully three-fourths of the progress in the control of uncinariasis is due to scientific research. One hardly dares to pick out those whose contributions have been most valuable, but these must be named: the Commission in the Orient, with Darling, Barker, and Hacker as outstanding figures, and later Cort, Grant, and Stoll in China; the Brazilian group with Smillie, Hackett, Darling, and Pessoa; the Home Country group with Maurice Hall, Kligler and Paul Lamson, in the United States; and lastly, and very especially, that notable West Indian group who began in Trinidad and wound up in Porto Rico, in Utuado, a name associated with more than one desperate struggle against hookworm in America, there literally to light the path to ultimate success in the eradication of hookworm disease. For only after achievement in Science can we talk of the eradication of a disease, "eradication" implying a purposeful act on the part of man in his war against Nature's inroads.

This modest little group of workers, living like poor farmers and working 18 hours a day, rapidly solved incredible problems, and gave material proof of the soundness of tenets grasped by instinct and epidemiological observation the world over. Not only does the allegiance of the writer go to this group of workers for sentimental reasons and for their scientific worth, but because he spent a while observing them in the midst

of their labors and came to sense their spirit of investigation.

But behind all these men and women towers the lonely figure of Baermann, a Dutchman of Java. This man furnished the lever which has raised practically every weighty obstacle from the path of progress. Baermann discovered a method by which the number of infective larvae could be counted in a given quantity of soil.

From this one contribution have come practically all the others. It was the door through which had to pass all of the forces which are now conquering uncinariasis. Baermann found that by half-submerging a sieve of earth in a funnel of water, all the larvae would leave the earth and collect in the stem of the filter. By drawing off and centrifuging the first few cubic centimeters, a basis could be found for estimating the infectivity of soil in a given locality.

As in all great working plans for the accomplishment of a sociological reform of a medical nature, the basis has been modestly built by patient delvers in science. Behind the solitary figure of Baermann rise the figures of Cort and his associates, one of them, a woman, Florence King Payne, whose contributions have made possible a practical working plan for a modern campaign against uncinariasis and have been of incalculable value in raising the efficiency of human agricultural labor and diminishing the cost of its accomplishment.

The precise knowledge, thus secured, has made it possible to ascertain, by research, such fundamental facts concerning the infection carrying larvae of the hookworm as the following: the time of survival of the larvae in the soil under various conditions of climate, of character of the soil, moisture, exposure to sunlight

or shade; the possibilities of migration in the soil under varying conditions; the lack of resistance to such a factor as acidity. Space permits only the briefest summary of some of these facts, which have contributed so much to the effectiveness of the war against this enemy. Sunlight and dryness of soil shorten the period of survival of the larvae, shade— even the shade of grasses and low plants —and moisture lengthen this period. Excess of water is detrimental to the life of the larvae. It is rare for them to live over three months in any soil and under normal conditions they begin to die rapidly after the first week or so, to disappear entirely in from six weeks to two months.

The larvae migrate vertically only, never laterally, and then can rise three feet or more vertically through favorable soil, for instance from buried excrements. Infestation of the soil is therefore practically limited to the point of soil pollution.

At the surface of the earth larvae are found undulating and upright on stones, sticks, particles of earth and dead leaves. In this exposed position they immediately "jump" a man's bare feet, attracted by the warmth of the feet.*

*Note.—The following references will prove of extreme value to anyone actively interested in the hookworm problem:

(1) Monographs Nos. 14 and 17 of The Rockefeller Institute for Medical Research, 1921 and 1922: "Studies on Hookworm Infection in Brazil," by S. T. Darling and W. G. Smillie.

(2) "Suggestions for the Mass Treatment of Hookworm Infection." *London Lancet*, 1920, II, 69.

(3) "The Treatment of Hookworm Infection," by S. T. Darling, M. M. Barber and H. P. Hacker. *Journal of the American Medical Association*, Vol. LXX, page 199.

(4) "Researches on Hookworm Disease in China," by W. W. Cort, J. B. Grant, N. R. Stoll and others. *American Journal of Hygiene*, Monographic Series, No. 7, October, 1926.

Thus ends, to date, the history of a great war, a world war. Of course, the entire world was unprepared for it and the first type of resistance was intensely local and limited to mines, all trench fighting, all defensive engagements. Then America took the field, took the offensive, first in Porto Rico, then in the Southern States, and finally in whatsoever part of the world the enemy could be routed out to fight. We are on the offensive and we are winning, and our chemical warfare has been intensified by two more valuable weapons —chenopodium and carbon tetrachloride. Most of the strategic positions of the enemy are ours today. Their complete conquest is only a question of time.

But we have totally lost sight of our enemies' allies, poverty and malnutrition, and if we keep on ignoring them they are simply going to give us trouble from another quarter. It is fitting that in closing we should merely refer to those deplorable nutritional deficiencies that form the background of many a slow-going tropical disease. Indeed, in sprue and even in uncinariasis in Porto Rico, nutritional unbalance is the problem; the parasite, the germ, a superimposed phenomenon. Why, after killing from 6,000 to 7,000 persons a year in 1899, did "anemia" in 1900 slaughter 12,000 and then fall to 7,000 or 8,000 deaths in the years following, before the uncinariasis campaign was even begun? For the simple reason that a fearful hurricane in 1899 destroyed the fresh food supply of Porto Rico and 5,000 cases of hookworm disease were turned into 5,000 fatalities by scantiness of proper food and consequent reduction of resistance to worms already harbored.

Why, after all, did people die in such large numbers

from uncinariasis in Porto Rico when it is well known that uncinariasis is not a potent cause of death in the Southern States? Chiefly because Porto Rico ate a ration whose chief components were rice, codfish, and unsubstantial tubers—at least that part of Porto Rico which suffered from hookworm disease.

Why do certain symptoms we used to think peculiar to uncinariasis, crowd to the fore in sprue, in chronic dysentery, in pellagra, yes! even in tuberculosis and pernicious anemia? Simply because they are really symptoms of the background of nutritional unbalance, enhanced by the superimposed influence of parasite or microbe.

The germ, the parasite, is responsible for the clinical picture we recognize as distinctive; the condition of the host upon which the parasite thrives is the major part of the therapeutic problem. This means that after we have exterminated the specific causal agent we may not have necessarily cured the patient.

These thoughts are left with those who care to listen to one whose twenty-five years in the tropics have taught him that, exclusive of the man-eating beasts, like plague, cholera, and yellow fever, the jackals get the men, women and children weakened by malnutrition.

The day is bound to come that a victorious Foundation will have a social service department the like of which the world has never before seen, and the correction of malnutrition due to poor food in the tropics will be its slogan.

Chapter IX—Article 8

THE COMBAT AGAINST SYPHILIS

ARTHUR S. LOEVENHART, M.S., M.D. and W.K. STRATMAN-THOMAS, Ph.D.

Professor of Pharmacology and Toxicology, College of Medicine, University of Wisconsin; and Research Fellow in Pharmacology, University of Wisconsin

Professor Loevenhart started his professional career in the Johns Hopkins Medical School and has been professor of Pharmacology and Toxicology at the University of Wisconsin since 1908. During the war he was chief of the pharmacological section of the research division of the Chemical Warfare Service. He is one of our leading American investigators in the study of the action of drugs and in the chemotherapy of syphilis, particularly of the central nervous system.

Dr. W. K. Stratman-Thomas has held an appointment as Research Fellow in Pharmacology with Dr. Loevenhart for the past four years. They have been studying the chemotherapy of trypanosomiasis (South African sleeping sickness). Dr. Stratman-Thomas has just received a John Simon Guggenheim Fellowship for continuing this work in tropical Africa next year, where clinical trials of several new drugs will be made.

"*War and Syphilis Are the Two Greatest Enemies of Mankind*"

No aspect of medicine gives a more striking example of the tremendous benefits to mankind resulting from a rational effort to find proper methods of treatment of disease than does the combat against syphilis. Syphilis is a specific disease, usually of slow evolution. Acquired syphilis is always characterized by a primary sore on the skin or mucous membranes of the body. Within two or three months constitutional symptoms develop which are characterized by skin eruptions and sores on the mucous membranes, sometimes with fever. After months or years a certain type of growth called a gumma may appear in any tissue of any part of the body. Finally,

sometimes after many years, the patient may suffer from syphilis of the central nervous system, diseases known as locomotor ataxia and general paresis.

The primary sore is the site where the organism causing syphilis enters the body and may appear anywhere. It is a sore with very hard edges and the lymph glands in the neighborhood become enlarged and hard. This primary sore is extremely slow in healing unless proper treatment is instituted.

In the secondary stage, there may be fever, anemia, jaundice, various types of skin eruptions, and sores on the mucous membranes, especially of the mouth. There may be a loss of hair and the eyes may become affected. The third stage, as stated, is characterized by growths called gummata, and the symptoms which they produce depend on their location; thus, a gumma of the brain would give symptoms of a tumor of the brain.

The congenital form of syphilis may be due to infection in the womb and the child often dies before birth. If the child lives, it develops certain characteristic stigmata, such as peculiar teeth, eye lesions, ear affections, changes in the bones, etc. As we have indicated, syphilis may affect any organ or tissue in the body, and for this reason Osler, one of the great clinicians, stated to his medical students, in substance, as follows: "Know syphilis in all its manifestations and all things clinical will be added unto you." In other words, if a physician is able to differentiate syphilis in all of its manifestations and forms from all other diseases, he must know the whole field of clinical medicine.

Syphilis has always had a special interest for thinking mankind because it affects not only the present but the following generation, and for this reason it was recog-

nized that the discovery of satisfactory treatment was extremely important. Free clinics and State control, as well as "enforced reporting," have been strongly advocated because of the great effects of the disease on the public health.

History of Syphilis.—The history of syphilis, which we can only touch upon here in the briefest way, is one of the most interesting subjects in the history of medicine. Whether syphilis existed in Europe previous to the year 1493 has never definitely been proved. After 1493 many tracts on the subject of syphilis appeared and for the first time it was recognized as a great scourge. It was called *Morbus Gallicus.* At the time of dispersion of the army of Charles VIII of France from Naples in 1495, and immediately following, the disease spread throughout Europe. The severity of the cases would lead one to believe that it was a new malady, since we know in general that new infectious diseases introduced into a community, before any immunity or resistance has been built up, always run a very virulent course. The fact that the disease made its appearance soon after the return of Columbus from his first voyage to America gave rise to the general belief, which has been widespread, that the sailors on this voyage contracted the disease in the western hemisphere and carried it back to Europe. While a great deal has been written on both sides of the question, it is exceedingly doubtful that this could have happened because of the very short interval of time between their return and the reference to syphilis in 1493. In the opinion of Sudhoff, the greatest living authority on the history of medicine, syphilis probably existed among civilized people from the very earliest times.

In 1530, Fracastorius published at Naples a poem of about 1200 lines entitled "Syphilis sive Morbus Gallicus." The hero of the poem is a herdsman named Syphilis, and from this the disease has taken its name.

The first three stages of syphilis were soon recognized, but the aspects of the disease referable to the central nervous system—viz., general paresis and locomotor ataxia—were even as late as 1894 characterized by Fournier as parasyphilitic, by which he meant that they were diseases prone to grow on syphilitic soil, but not actually syphilis. Erb of Heidelberg was the first to collect sufficient data to indicate that they are probably late manifestations of syphilis. In 1905 Schaudinn and Huffman discovered the organism responsible for syphilis and called it *spirocheta pallida*. Schaudinn later renamed the organism *treponema pallidum*. Seen under the microscope in a drop of serum from the syphilitic sore of an infected animal, it appears as a small, cork-screw-shaped organism, and it is rather of animal (protozoön) than of vegetative (bacterial) character. With the discovery of the organism, it became possible to make an absolutely certain diagnosis of the early syphilitic lesions. Bordet and Gengou, in 1900-01, discovered an important method of determining the presence of certain bacterial diseases from an examination of the blood serum. In 1906 this was modified somewhat by Wassermann who applied it to the diagnosis of syphilis, and the reaction became known as the Wassermann reaction. This is a great laboratory help in the diagnosis of obscure cases. Noguchi and Moore, in 1913, found the treponema pallidum in the brain in cases of general paresis and this, together with sub-

sequent work, has proved beyond all doubt that paresis is simply a late phase of syphilis in man.

Early History of the Treatment of Syphilis.—The first successful treatment discovered was mercury in its various forms. In the first printed tracts on syphilis appearing in 1495-1498, mercury is mentioned in the treatment but it is doubtful whether it was used very largely in the first two years of the outbreak. The better practitioners used mercury exceedingly vigorously, often causing mercurial poisoning, salivation, loosening or loss of the teeth, together with many other manifestations of mercurial intoxication. This vigorous treatment caused a prompt reaction against mercury and various herbs were used in its place, together with other general measures which had no effect on the infection. The prompt use of mercury was probably due to the fact that one of the principal manifestations of syphilis is the skin rash, and mercury had been used successfully in certain skin diseases. The common method of administering mercury was by applying it to the skin in the form of an ointment, with the patient before a hot fire, and rubbing until the ointment disappeared. During the 17th and 18th Centuries, mercury was used with more moderation. Early in the 19th Century, mercury was again used more vigorously and again gave rise to a reaction in favor of the non-mercurial treatment. In 1821 Coindet advised the use of iodine in syphilis and iodides of starch and of iron were used in France and Germany but without success. In 1836 Wallace published brilliant results from the use of potassium iodide in doses of 2 gm. per day in 142 cases of constitutional syphilis. From this time on until the modern work, mercury and potassium iodide were the two great drugs

on which physicians depended in the treatment of cases of syphilis, and they are still a very important part of the doctor's methods of combating this disease. Potassium iodide is only of service in the third or gumma stage; it causes these growths to melt away in a very remarkable manner. Mercury may be used with advantage in all stages of syphilis.

Moral Aspect.—The moral aspect of syphilis requires a brief word. It should be distinctly understood by all that syphilis is by no means always a venereal disease. The disease is often contracted without any implication whatever of immoral conduct and is purely a chance infection, such as occurs in typhoid fever and many other diseases. Vast numbers of such cases are seen where it is easy to prove that the disease was contracted extragenitally. It should be further understood that the doctor is not concerned with the moral aspects of the disease. When he sees a syphilitic patient, his concern is only with the individual's health and with the protection of the people with whom the patient comes in contact. Yet, the moral implications often influence the patient's conduct and cause him to endeavor to keep his infection secret.

There has been a great effort made in recent years to decrease the incidence of syphilis and this can be most effectively accomplished by adequate treatment early in the infection. This is an instance in which curative medicine is the best means of prevention of the disease. In other words, the best way to prevent syphilis is to render patients non-infectious in the shortest possible time. Fortunately synthetic organic chemistry has put into our hands some excellent new curative agents to bring about this result, as we shall presently see. Pre-

ventive medicine is often represented as more important than curative medicine; as a matter of fact, in many cases they are so closely interwoven that it is impossible to separate them and this is preeminently true in the case of syphilis.

The incidence of syphilis has been much discussed. It may be conservatively stated that ten per cent of the community has a syphilitic taint. The economic loss due to the disease from the standpoint of decreased earning power, cost of hospitalization, medical care, as well as premature death, is exceedingly difficult to estimate and we must, therefore, be content with the bare statement that it is enormous.

Modern Chemotherapeutic Work on Syphilis.—This phase of the work had to wait upon the development of methods of producing the disease experimentally in animals. Metchnikoff first succeeded in infecting apes with syphilis; later a method of infecting rabbits was devised, and the rabbit has remained the principal animal for work in experimental syphilis. It again emphasizes the tremendous importance of animal experimentation in all medical advance. Those who know the importance of animal experimentation for the investigation of disease and its proper cure are constantly annoyed with the fanatical opposition of a small group of people, who lead such happy lives that they never come in contact with anything more pitiable than a dumb animal, and who have no conception of the mass suffering of the race, or are totally callous to it.

The term "chemotherapy" is somewhat difficult to define. It was introduced by Erhlich and the definition and field of work indicated by the term can be best illustrated by the work done in Ehrlich's laboratory on

syphilis and trypanosomiasis. Chemotherapeutic work on syphilis was an outgrowth of the work on trypanosomiasis, and for this reason we must briefly discuss trypanosomiasis at this time. Under the term "trypanosomiasis" we include a series of diseases of man and animals which are produced by organisms related to the organism causing syphilis. These diseases, such as African sleeping sickness, are of tremendous importance in the world and will be referred to later on in this paper. Trypanosomal infections are of importance in connection with the chemotherapy of syphilis because they can be given to animals very readily, and the animals die promptly unless effective remedies are administered. Thus, the duration of life of rats in many forms of trypanosomiasis is only three to nine days. whereas experimental syphilis runs an extremely chronic course.

Ehrlich's views regarding chemotherapy were based on the general proposition that certain organic chemicals have a specific affinity for certain living cells, and the term was an outgrowth of Ehrlich's views regarding the nature of immunity. We would define chemotherapy as that phase of pharmacology which deals with the relation between the chemical structure of therapeutic agents and their effect on living things. Ehrlich used the term only in reference to infectious diseases, but there is no reason why it should not include all types of substances used in therapeutics.

In practice, work in this field has always started with a fortuitous discovery that a given organic chemical has a certain specific pharmacological action. Following this lead, various derivatives of the drug are then prepared in the hope of finding a substance which is less toxic to

man and more potent in the desired type of pharmacological activity than the original substance. The production of derivatives is not done in a routine or haphazard manner. We have accumulated a certain body of fact regarding the biological effect of certain groupings and the derivatives are made in accordance with past experience. In the case of materials used in the treatment of infections, chemotherapeutic studies are directed toward the production of substances highly toxic to the invading parasite and of low toxicity to man and the higher animals. The whole field of chemotherapy is one of the most promising for medicine of the future. It is on the border-line between chemistry and medicine and requires the cooperative effort of chemists, pharmocologists, and clinicians. The particular phase of chemistry concerned in most cases is synthetic organic chemistry and the work must be carefully coordinated in order to be successful.

The Earlier Arsenicals.—In 1863 Béchamp prepared a substance by the action of arsenic acid on aniline, which he thought to have the formula $C_6H_5NH.AsO(OH)_2$. He also prepared several salts of this acid. The sodium salt was first used therapeutically by Schild and Kionka in 1902 and the first animal experimentation with the substance was done by Blumenthal in the same year. They were interested in the sodium salt of this acid from the standpoint of general arsenical therapy, and not from the standpoint of the treatment of syphilis or trypanosomiasis. Blumenthal found it much less toxic to animals than the inorganic forms of arsenic and this relatively low toxicity caused the adoption of the name "Atoxyl" for the substance.

The early literature of the last century contains references to the clinical use of arsenic in inorganic form in the treatment of trypanosomiasis. Laveran and Mesnil, in 1902, studied the action of inorganic arsenical compounds in experimental trypanosomiasis and found them somewhat effective in that they checked the infection but never cured the disease. Thomas, in 1905, first studied the action of atoxyl in experimental trypanosomiasis and found it to be an effective curative agent. Robert Koch, in 1907, showed that in clinical trypanosomiasis, atoxyl causes the organisms to disappear from the blood stream; and from that time up to the present, atoxyl has continued to be used clinically in the treatment of trypanosomiasis in the human.

Ehrlich began his chemotherapeutic studies on the arsenic compounds with atoxyl. At the beginning he studied the effect of atoxyl on trypanosomes in the test tube and found that it was not effective; even in high concentrations it did not kill the organisms. For this reason he temporarily discontinued work with it. Later Ehrlich again studied the action of atoxyl in the living animal infected with trypanosomes and confirmed the observations of Thomas, that it is very effective. Several investigators have shown that blood or tissue extracts, when treated with atoxyl, yield a substance which kills trypanosomes in the test tube. It was evident therefore that atoxyl has to be altered in the body before it is capable of destroying these organisms. Following the atoxyl lead, Ehrlich and his coworkers prepared a very large number of derivatives of atoxyl. So far as we have been able to find, a complete list of the substances prepared and studied by them has never appeared. Ehrlich

and Bertheim later made a study of the structure of atoxyl and found that instead of being of the nature of an anilide, it is really para-amino-phenylarsonic acid,

$$H_2N.C_6H_4.AsO(OH)_2$$

In their monograph on chemotherapy, Ehrlich and Hata mention specifically relatively few compounds. It soon became evident from the clinical work on atoxyl that the drug produces serious injury to the eyes in a considerable number of cases, occasionally causing complete and permanent blindness. The first attempt to modify atoxyl and to reduce its toxicity resulted in the production of its acetic acid derivative, arsacetin,

$$CH_3CO.NH.C_6H_4.AsO(OH)_2$$

This substance continued to be used clinically in the treatment of trypanosomiasis for many years, although it had just as severe an effect on the optic tract as atoxyl and possessed no advantages over it.

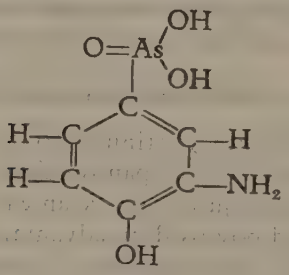
Among the large number of derivatives produced by Ehrlich and very extensively studied by him and his coworkers was arsenophenylglycine,

$$HOOC.CH_2NH.C_6H_4.As{=}As.C_6H_4.NHCH_2COOH$$

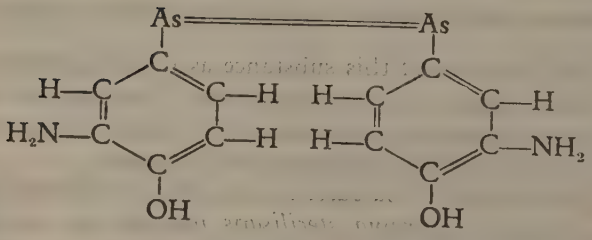
Ehrlich regarded this substance as highly satisfactory in the treatment of trypanosomiasis. The basis for this statement was that animals were cured of trypanosomiasis by a single treatment with the drug.

Arsphenamine (*Salvarsan*) *or* "606."—Ehrlich had the dream of *therapia sterilisans magna*—i.e., a drug which would produce a complete sterilization in a single

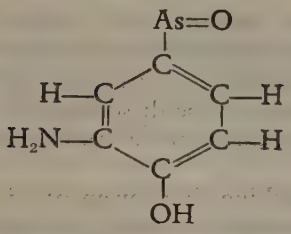
dose, both in syphilis and trypanosomiasis. In regard to syphilis, certainly, this dream has never been realized, and in regard to trypanosomiasis it has only been realized in the case of the smaller animals such as the mouse, rat, and rabbit. There are three types of organic arsenicals which have been studied especially in syphilis and trypanosomiasis. They are (1) the arsonic acid, (2) the arseno, and (3) the arsinoxide types. We may illustrate all three of these types of compounds with the nucleus of arsphenamine, as follows:



1. Arsonic Acid



2. Arseno form (Arsphenamine)



3. Arsinoxide

Arsphenamine was finally accepted by Ehrlich as the most favorable of all the compounds which he studied in the treatment of syphilis; no substance approaching it in value in the treatment of the acute aspects of syphilis has been since discovered. Ehrlich was so pleased with its effects and so convinced that the new drug would be the salvation of mankind by destroying one of its greatest scourges, that he named the drug "salvarsan"; it also became known as "606," under which name it was recorded in Ehrlich's laboratory in the long list of arsenicals prepared for study. Its pharmacopœial name is arsphenamine.

The substance was placed on the market in vacuum sealed ampules in the form of the dihydrochloride in 1910. Very remarkable clinical effects were reported at once and the discovery startled the world; it was thought that this dread disease had been vanquished by one stroke. Many cases of syphilis with most distressing lesions, which had received mercury and iodides, yielded immediately to arsphenamine and healed. It was soon found, however, that one treatment could not cure and that it requires great persistence to effect a cure of syphilis in man in most cases. This compound is readily soluble

in water but, on account of its acidity, is unusable in this form. Four equivalents of caustic soda must be added before it is ready for injection into the vein in the form of a solution of the di-sodium salt. To inject the unneutralized material into a vein would cause almost instant death.

The most striking improvements in arsphenamine have been in methods of making it more easily soluble. Thus, Ehrlich's laboratory also produced neoarsphenamine,

$$NaOSOCH_2HN.C_6H_3(OH)\,As{=}AsC_6H_3(OH).NHCH_2OSONa$$

a product of the action of sodium formaldehyde sulphoxylate on the amino group of arsphenamine. It is a great improvement from the standpoint of convenience to the physician because it is unnecessary to neutralize this product prior to injection. The contents of the ampule are simply dissolved in distilled water and may be injected directly into the vein with the use of a hypodermic syringe. Neoarsphenamine can also be given in a more concentrated solution than arsphenamine. It has certain advantages and certain disadvantages. It is less toxic than arsphenamine and is less likely to produce severe reactions. It also has the advantage that it effects a more prompt healing of active syphilitic lesions. Apparently the reason is that the substance is less colloidal, it penetrates into the tissues more readily, and it seems to increase the resistance of the tissues to the presence of the treponema pallidum. Its disadvantages are that it is less treponemicidal; it is more unstable chemically; it is not such a definite chemical substance on account of the indefiniteness of the formaldehyde sulphoxylate group, and a larger dose is required than

of arsphenamine, although the margin of safety—i.e., the therapeutic index—is at least as great.

Ehrlich was the first to introduce the term "therapeutic index," and by this term he meant the ratio of the toxic dose to the therapeutic dose. It should be pointed out that the therapeutic index, as determined from experimentation with small animals, often does not hold for man and the larger animals. Thus, Bayer-205, a non-arsenic-containing product, offered for sale under the name of "Germanin," has been a great disappointment in the treatment of human trypanosomiasis in spite of the fact that it has a very high therapeutic index for mice. Similarly, neoarsphenamine has a very high therapeutic index in trypanosomiasis in rats, but it is of no value in the treatment of human trypanosomiasis.

Another closely related derivative of neoarsphenamine is a preparation called sulpharsphenamine, produced by Voegtlin, Johnson and Dyer in the Hygienic Laboratory of the U. S. Public Health Service. It has the following structure:

$$NaO_2SOCH_2NH.C_6H_3(OH)As{=}AsC_6H_3(OH).NHCH_2OSO_2Na$$

This product, although very similar chemically to neoarsphenamine, differs from it somewhat in its biological effects and may have advantages over it in the treatment of certain syphilitic lesions.

As stated previously, Ehrlich observed that atoxyl does not destroy trypanosomes in the test tube. He also observed that the arsphenamines are not effective in the test tube. On the other hand, the arsinoxide compounds (formula 3, p. 667) corresponding to these substances

are effective outside the body. Voegtlin and Smith showed that when the arsonic acids or the arsphenamines are injected into rats whose blood swarms with trypanosomes, there is a certain latent period varying from one to three hours before the trypanosomes begin to disappear from the blood. On the other hand, when the corresponding arsinoxides are administered, the trypanosome count begins to be reduced at once. They very logically conclude from this work that both the arsonic acid and the arseno types of compounds have to be changed in the body into the corresponding arsinoxides before they become effective. The latent period observed before the organisms begin to disappear can be most readily explained as due to the time required to build up an effective concentration of the arsinoxides. It has been found by all investigators who have studied the question that in therapy the arsinoxides cannot be used as successfully as the other types of compounds. The relative parts played by the parasitic organisms and by the cells of the host in the reduction of the arsonic acid compounds, or in the oxidation of the arseno compounds, may account for the fact that the drugs can be best administered in the inactive forms and permit them to be activated by the living body.

Stovarsol is the acetyl derivative of the arsonic acid corresponding to arsphenamine. It has the formula:

$$CH_3CONH.C_6H_3(OH).AsO(OH)_2$$

It was prepared by Ehrlich and his collaborators. This drug has been used extensively in France in the treatment of syphilis and is undoubtedly far less effective than the arsphenamines. The increase in syphilis

which has been noted in France during the last few years has been attributed by some writers to the fact that stovarsol has been very largely substituted for the arsphenamines, with the result that the patient remains longer in a state in which he can infect others.

It seems highly probable that other drugs might be found which would be more effective than the arsphenamines in the treatment of active syphilis. The drugs sometime produce very disagreeable, and occasionally even fatal disturbances and, in the opinion of the writers, work should continue in an effort to find still more effective drugs in the treatment of the various manifestations of acute syphilis.

Syphilis of the Central Nervous System—General Paresis; Tryparsamide.—General paresis is one of the most distressing and fatal forms of mental disease. In the treatment of this condition, the arsphenamines proved themselves to be of very little value. In a systematic study of the chemotherapy of neurosyphilis by Lorenz, Loevenhart, Bleckwenn and Hodges, it was determined to study the action of drugs in the treatment of this condition irrespective of their effectiveness in the earlier stages of syphilis. Among the substances studied by them was tryparsamide,

$$H_2NCOCH_2NH.C_6H_4.AsO(OH)_2$$

This substance is the amide of the arsonic acid which had been previously studied by Ehrlich in the arseno form: viz., arsenophenylglycine. It was first produced at the Rockefeller Institute for Medical Research by Jacobs and Heidelberger and studied experimentally in animals by Brown and Pearce in the same Institute. It

was found by Lorenz and his coworkers that this drug is exceedingly effective in the treatment of general paresis and resulted in the mental restoration of 40 per cent of the patients who had been committed to state institutions, enabling them to return to their families and to resume their work, often with increased earning capacity. This important result has been confirmed by a very large number of investigators. Tryparsamide has a remarkably interesting tonic effect and the patients frequently gain from ten to thirty pounds in weight during the treatment. The mental restoration of the patient is often quite sudden and they can often fix the time, to a day, when they became mentally clear. When asked what happened, they state that "the veil lifted" or "the mist rose" which had separated them from the real world about them and had shut them in.

Another treatment of general paresis which has nothing to do with chemotherapy, but which is exceedingly interesting and effective, is the infection of the patient with malaria and, after a certain number of chills, relieving the malarial condition by the use of quinine. This was introduced by Wagner-Juareg, a Viennese clinician. This form of treatment is considerably more hazardous than that by tryparsamide, the mortality being about ten per cent. The disadvantages of the malarial treatment are sufficiently obvious to need no comment.

Tryparsamide also has the disadvantage that in one to two per cent of the cases, especially where there is involvement of the spinal cord, there occurs a more or less serious impairment of vision which has to be carefully watched. It is in this regard in particular that there is a necessity for finding a better drug than tryparsamide.

Other Drugs Used in the Treatment of Syphilis.—Mercury and iodides continue to be valuable adjuncts in the treatment of certain types of syphilis. There has been no great improvement in the use of these drugs. A great deal of work has been done on the chemotherapy of mercurial compounds but no practical advance has thus far been made, although the synthesis of new mercurial compounds offers a very promising field in the treatment of syphilis. Various compounds of bismuth have recently been introduced and they are undoubtedly of value in the treatment of syphilis, especially in cases which have a peculiar sensitiveness to the arsphenamines, but bismuth compounds are by no means as effective as the arsphenamines. It is possible that bismuth compounds may in the future replace mercury to a certain extent.

The arsphenamines have been found to be exceedingly valuable in the tropical disease known as frambesia or yaws, which is very prevalent in certain districts. It seems as though this disease could be completely wiped out by the extensive use of the arsphenamines. Arsphenamine is also valuable in the treatment of relapsing fever and has also yielded favorable results in the treatment of Vincent's angina. The organisms responsible for these conditions are more closely related to treponema pallidum than to the trypanosomes.

Trypanosomiasis—African Sleeping Sickness; Tryparsamide.—Trypanosomiasis still presents a great world problem. Approximately one million square miles of equatorial Africa is affected by trypanosomal diseases, which in man cause the African form of sleeping sickness, and in animals cause the disease known as *nagana*. African sleeping sickness is an entirely different

disease from encephalitis lethargica—the so-called "sleeping sickness" occurring in this country. The African disease has at certain periods caused the majority of deaths in certain regions and has decimated the population. It also attacks all of the domestic animals. Tryparsamide was shown by Brown and Pearce to be exceedingly effective in the treatment of experimental trypanosomiasis, and Pearce showed that it is the most beneficial drug known in the treatment of human trypanosomiasis, especially in the late stages when there is involvement of the central nervous system. Investigators have found tryparsamide to cause the same remarkable mental restoration that had been previously noted by Lorenz and his coworkers in general paresis. The work of Pearce has been amply confirmed and there is no doubt that it represents the greatest advance in the treatment of human trypanosomiasis that has been made thus far. Here again, it has been observed that tryparsamide, in a certain percentage of the cases, deleteriously affects the vision, and it is especially from this standpoint that further work needs to be done to develop drugs which are safer from this viewpoint. It has been found that tryparsamide is not so effective in the treatment of the form of South African sleeping sickness caused by *Trypanosoma rhodesiense*. The more common form which yields to tryparsamide is caused by *Tr. gambiense*. The drug has also been found to be quite ineffective in the treatment of certain forms of trypanosomal disease attacking horses and cattle, such as the disease known as *surra*, which is prevalent in the Philippine Islands and causes enormous economic loss.

We have indicated that experimental trypanosomiasis has played a great rôle in chemotherapeutic studies of

syphilis. It should be pointed out, however, that there is by no means a parallelism between these two diseases, since drugs are known which are very effective in syphilis and totally inert in trypanosomiasis, and the converse is also true.

While we have been able only to give the briefest outline of the history of the combat against syphilis, it must show a most hopeful outlook for ultimate victory over the disease and, in time, possibly its complete suppression. This great advance in the treatment of the disease would not have been possible without the aid of creative chemistry. So great indeed have been the results of the cooperation of medicine and chemistry in this field that the fighters against other diseases have been heartened and encouraged to follow the plan of campaign that was initiated by Ehrlich.

CHAPTER IX—ARTICLE 9

THE BATTLE AGAINST TUBERCULOSIS

ESMOND R. LONG, PH.D., M.D.

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Dr. Long was a Trudeau Fellow at the Saranac Laboratory for the Study of Tuberculosis in 1917-18, and in 1920. In 1911-13 and since 1919, he has been on the staff of the Department of Pathology of the University of Chicago. Dr. Long has devoted more than ten years to an intensive study of the chemistry and immunology of tuberculosis. His work has been along fundamental lines and has received not only widest recognition but also generous support from the National Tuberculosis Association. He is the co-author of *The Chemistry of Tuberculosis* (1923).

Tuberculosis has always been one of the most serious diseases of mankind, and investigators in many scarcely related sciences have cooperated to find some means of curbing its progress. Chemists have made notable contributions to our understanding of the disease, if they have not yet furnished a means of direct attack, as in the case of a number of other physical ailments.

Consumption has long been known to be an infectious disease, one caused by a small germ, the tubercle bacillus, that can be cultivated on special nutrient media outside the body. All chemical study takes this fact into consideration. The problem of controlling the disease is essentially the problem of inhibiting the growth of this germ within the body. Preventing growth within living tissues, however, is so much more difficult than killing the germ within the test tube, as to demand approach at the problem from many angles.

Three of the most hopeful lines of attack, by no

means the only possible ones, will be considered briefly here, viz., "specific chemotherapy," study of the chemical relationship of germ and patient, and investigation of the chemistry of the germ itself.

The Specific Chemotherapy of Tuberculosis.—Theoretically one of the simplest conceivable ways of stopping the course of an infectious disease is the administration of a substance highly poisonous to the microbial cause of the ailment, but relatively non-poisonous to the tissues of the human being infected. This is not fanciful or by any means impossible of achievement. It is well known that there is often a wide divergence in the susceptibility of different animals to one and the same poison. This knowledge led to the fruitful work of Ehrlich on the treatment of trypanosome infections and syphilis. With initial success in this field came the development of a new science, "specific chemotherapy," in which the effort is made to find chemical substances more injurious to the bacterial cause of the disease under consideration, and less injurious to the organs of the individual. A mathematical ratio, called the "therapeutic index," has been set up, taking into account the relative susceptibilities to the drug of a given germ, on the one hand, and animals suitable for laboratory infection with it, on the other. Drugs with high killing power for germs and low killing power for animals have been eagerly sought since the first successful efforts of Ehrlich and his associates; and in the case of certain diseases particularly the tropical infections by trypanosomes, great success has attended the effort.

Ehrlich's original ideal was a drug so potent against the germ of a disease and at the same time so little injurious to man, that a single dose would literally dis-

infect the patient as far as the particular germ was concerned, without injuring any of the healthy tissues of the body. Results even in the most successful fields of chemotherapy, however, have fallen far short of this ideal, and physicians today would be content in most instances to know of drugs which on repeated administration would keep the growth of the germ inhibited until the natural forces of the body effected the final killing.

Even this far less ambitious program has not been satisfied in the case of tuberculosis. Failure has not been for lack of effort, however. Fortified by the patience of Ehrlich and his coworkers, who investigated more than six hundred compounds before they were at all satisfied with their results in syphilis, numerous investigators have valiantly pursued tedious and inconceivably laborious programs directed at the germ of tuberculosis.

These programs in general have been more comprehensive in scope and more scientific in plan than the original experiments on syphilis, in which a lucky start and a gambling faith on the hit-or-miss method, had much to do with the successful conclusion. In tuberculosis account has been taken from the start of the complexity of the tuberculous lesion itself, and of its characteristic features as they might affect the penetration and action of drugs highly poisonous to the germ of tuberculosis outside the body.

Investigation of the possibilities of chemotherapy in tuberculosis properly begins with the study of the body afflicted by the disease, and many precious results have been secured in the latter field by chemists all over the world, who have been content to record their discoveries in scientific literature and leave them until the proper

time and proper men arrive to make practical use of them. Put together these isolated chemical discoveries are building a foundation secure enough to support scientific precision in a direct attack on the tubercle bacillus.

The best conducted chemotherapeutic experiments on tuberculosis today consider the growth-inhibiting as well as true disinfecting power of drugs, within as well as without the body. They take note not only of the degree of toxicity of the drug for animal cells, but also its ease or difficulty in penetrating the actual lesion of tuberculosis within which the infecting germs lie. It was early discovered that certain dyes penetrate the limiting layers of tuberculous lesions rather readily, and this finding led to the hope that disinfecting metals, such as arsenic or mercury, might be chemically combined with these dyes without changing their penetrating power, and sent into the body in this form. It was soon found that metal-dye combinations could be produced, the toxicity of which for animal tissues was vastly below that of the free metal. It was the original hope in many an experiment that such a compound could be given by injection or otherwise, and allowed to accumulate in the specific lesions of tuberculosis, there to undergo slow disintegration with liberation of the poisonous metal in concentration strong enough to destroy the germs in the affected part.

A most disconcerting discovery was that poisonous metals can occasionally accumulate in tuberculous lesions until present in concentrations easily high enough to kill germs in the test tube, without destroying them in the lesion. This finding was only another reminder of the necessity of knowing the conditions operating within

the lesion itself. The study of chemotherapy in tuberculosis cannot afford to neglect the chemistry of the tuberculous body. If a disinfecting material can accumulate in a tuberculous lesion in large quantity without destroying the germs present, the latter must be protected in some way. To ignore this fact and push blindly on in the injection of compounds found highly poisonous to the germs in the test tube, would be most wasteful of time, effort and money.

As a matter of fact the manner in which the protection is afforded is constantly becoming better understood. It has come to be appreciated that body cells engulfing bacteria may protect these from outside destructive agencies, instead of invariably destroying them rapidly in the way so frequently emphasized. Thus penetration of the gross lesion of tuberculosis is not enough. There is the still more difficult problem of penetrating certain live cells, known for the selective way in which they pick up or refuse particles of different sorts.

Recent investigations also on the tendency of tubercle bacilli themselves to penetrate fat droplets and lie in their center, may furnish the explanation for many puzzling phenomena. Among other things they may throw some light on physical factors protecting the tubercle bacillus in tuberculous lesions.

Thus the field of chemotherapy is a very broad one requiring work in many quarters. It must be recognized that our knowledge of the chemistry of the tuberculous body lags behind our understanding of the manufacture of germ-destroying chemical compounds. Not many of the brilliant chemists laboring successfully in the latter field have been attracted to the more difficult problems

of the former. What progress has been made in the harder field has for the most part been accomplished by men stubbornly determined to investigate the problem, but often, unfortunately, insufficiently trained to follow up their hard won achievements. It is scarcely questionable that the chemotherapy of tuberculosis in its broadest aspects offers a promising field for the well trained chemist.

Chemical Relationships of Germ and Patient.—Many investigators feel that a thorough understanding of the life habits and food requirements of the germ will ultimately enable us to balk its multiplication in the human body. Consequently much investigation has been devoted to the manner in which the germ "breathes," the food elements that it must have, and the limitations set by the accumulation of its own excretory products upon its further growth.

While there has not been perfect agreement upon all the details of the respiration of the tubercle bacillus, it is known that it requires oxygen at a pressure not far from that most suitable for our own development, approximately, that is, within the range of atmospheric air, and that like ourselves it gives off carbonic acid gas, which in sufficient concentration becomes harmful. Some very good observers have claimed that when the concentration of this gas in the air surrounding growing tubercle bacilli passes six per cent growth stops. This has been disputed by other investigators. It offers such a plausible explanation, however, for the failure of growth of the germ in certain places in the human body, when at the same time growth elsewhere in the same body is luxuriant, as to justify the most painstaking pursuance of the problem.

For example, tuberculosis practically never develops in muscles, the organs giving off the largest amounts of carbonic acid gas. It has long been believed that tuberculosis has somewhat less tendency to develop in lungs congested with poorly oxygenated, venous blood, as is the case in certain kinds of heart disease, than in normal lungs, and while the difference in susceptibility to tuberculosis is now known not to be so great as was once supposed, it is still large enough to suggest that the venous blood, rich in carbonic acid, may be inhibitory to the growth of the germ, at least in connection with other factors. In fact a method of treatment of localized skin and bone tuberculosis, based on retaining venous blood in the part, thereby excluding fresh arterial blood, has had some success. Other arguments could be advanced in favor of this theory on the relation of the accumulation of carbonic acid to the growth of the tubercle bacillus, so that altogether it seems a theory most worthy of further investigation.

In general the food requirements of the germ are very simple. They are so much more simple than those of the human body as to make it seem at first thought probable that any nutritional curtailment sufficient to impede the development of the microbe would be still more serious for the human organism. The newly discovered and still mysterious substances the vitamins, so important in animal and human nutrition, are unnecessary for the growth of the tubercle bacillus. Nitrogen, necessary to all forms of life, is readily assimilated by the germ of tuberculosis in the simple form of ammonia. The more complex amino acids essential to the development of the human body are equally suitable for the growth of the tubercle germ, but not necessary.

The mineral requirements on the whole are approximately the same as those of the human host, so that there is little chance of starving the germ in that respect.

On the other hand there is a more vulnerable spot in its carbon metabolism. All ordinary forms of life, man and the microbe of tuberculosis included, must have carbon in some form. They use it to build their fats, sugars, proteins and other substances necessary to existence, as well as for the production of energy. Human beings make use of carbonaceous matter in a great variety of forms. The great majority of bacteria are still less particular as to the source of their carbon. Curiously enough the tubercle bacillus stands almost alone in its dependence on one or two particular combinations of carbon. The most usable source of carbon by far is the relatively simple substance glycerol (pure glycerine). Combinations of nutritive substances for the tubercle bacillus may be perfect in every other respect, but, at least in laboratory culture, luxuriant growth does not occur in the absence of glycerol. In laboratory nutritive media, unless this substance is present in a concentration of at least one-half of one per cent, growth is scanty or fails to occur. The best and almost only substitute is some simple form of sugar, and even with glucose, probably the most suitable of these, growth is never so good as with an adequate supply of glycerol.

Glycerol, however, is always present in the human body. Body fat contains about five per cent and even in the leanest body at least an ounce or two is potentially available. It is important to note, however, that it is only potentially and not immediately available. The majority of the glycerol in the body is not free but

combined in the form of fat. In this form the bacillus finds great difficulty in making use of it.

Although the proof is still forthcoming, it may be that a difference in the availability of free glycerol in the tissues accounts for some of the differences noted in people in susceptibility to tuberculosis. There is a great deal of evidence that such difference in glycerol availability exists, although here again the direct proof is lacking. Suggestive evidence comes from the fat metabolism of the human body. It is a well known chemical fact that under certain conditions fats, which consist of fatty acids combined with glycerol, have a tendency to break up into their constituents, while under other conditions the constituents tend to recombine to form the original fat.

The actual conditions at work in this "reversible reaction" in the human body must be very complex, but it is known in a general way that some people have a strong tendency to lay up fat, while others, who may be equally healthy, are unable to put on much fat, regardless of how much they eat. It is thought that a more active oxidation is concerned in the second group. At any rate, according to the general belief, before oxidation of the food fat occurs, it is split into its component parts, viz., glycerol and fatty acids. The thin-inclined, who burn their fat readily, would therefore be expected to have at a given time more glycerol in the free state than the fat-inclined, and this factor might well influence the growth of tubercle bacilli.

In favor of this hypothesis—and it is purely hypothetical so far—is the fact that, in general, people who have a tendency to lay up fat rather than break it down, are less susceptible than thin people to tuber-

culosis. We must be cautious, however, before pushing this reasoning far, for tuberculosis is so insidious in its onset that thinness may be the *result* of an already existing tuberculosis, rather than a predisposing cause of the tuberculosis that eventually breaks forth.

There are some diseases, considered as predisposing to tuberculosis, in which the nutritional factor just outlined may be at work. Chief among these is diabetes. The low resistance of the diabetic to tuberculosis has usually been attributed to the excessive glucose content of the blood and tissues. As we have seen, glucose is one of the carbonaceous substances which can be used by the tubercle bacillus. However, in culture tubes in the laboratory, glycerol favors the growth of the germ much more than glucose. If either is concerned to unusual degree in the tuberculosis of the diabetic, it is probably the glycerol. The diabetic has a very active, although abnormal, fat metabolism, and possibly, therefore, an excessive quantity of glycerol in the free state.

Enough has been said to indicate that a thorough investigation of the availability of glycerol in the body is a desirable piece of tuberculosis research. Investigations on the problem are indeed under way. The great difficulty has been the lack of suitable quantitative chemical methods for the measurement of glycerol in blood and animal tissues. In the last analysis, therefore, the problem resolves itself into a purely chemical one. Should the theory briefly outlined be upheld by actual experiment it should be quite within the bounds of possibility to control the excessive free glycerol by a nutritive program based on well known chemical laws relating to reversible reactions.

The Chemistry of the Tubercle Bacillus.—A great deal of valuable information explaining some of the features of the disease has come from a study of the chemistry of the germ. A study of human tissues diseased with tuberculosis shows three main types of change from the normal, viz., "necrosis" or death of tissue, "exudation" or outpouring of blood plasma and special cells, and "proliferation" or formation of new tissue with a tendency to wall in the lesion and its microbial cause.

All these types of change can be reproduced through the use of purified chemical products isolated from the dead germ. In other words, while the progression of tuberculosis as an active disease depends on the multiplication and spread of tubercle bacilli in the body, the individual lesions of the disease, the nodular "tubercles," the fluid effusions, etc., are the result of action on the healthy tissues by chemical substances found in the bodies of the germs of the disease.

Among these chemical fractions of the tubercle bacillus are its fats and fat-like substances. About one-third of the dry weight of the germ is material of this character. Most of this in turn is not true fat, but a wax of a fairly well known chemical type noted for its great resistance to digestion and solution. There are good grounds for believing that this inert material is responsible for some of the accumulation of body cells which forms the "tubercle" or characteristic unit lesion of tuberculosis. This accumulation of cells, through local pressure on the blood capillaries, shuts off some or all of the blood supply to small regions, and "necrosis" or death of tissue results.

A much more important fraction of the tubercle bacillus, however, is its protein. There is little doubt that in the disease tuberculosis this protein plays a great part. Tuberculosis is one of those diseases in which the patient becomes "sensitized" to the cause of the disease, in this instance the tubercle bacillus. For a long time it was not well understood to what particular chemical fraction or fractions of the germ the patient was sensitized. Now, as a result of extensive investigations on the chemistry of the germ itself and products derived from it, found in the culture medium on which it has grown, it is quite evident that tubercle bacillus protein and protein derivatives are the substances concerned.

There is much reason for believing that the reaction of the sensitized tissue of the tuberculous patient to the protein of his own germs, is responsible for some of his anatomical disease changes and symptoms of illness. If a very small amount of purified tubercle bacillus protein is injected into a healthy-appearing part of a tuberculous animal, a violent reaction ensues, manifested to the unaided eye by redness and swelling. Applied to the skin this constitutes the well known tuberculin test of men and animals.

With the microscope a great deal more is seen. Essentially the changes are similar to those found in acute tuberculosis without much of the proliferative element, which, as we have seen, is partly due to the wax in the tubercle bacillus. In all probability the tuberculous changes occurring in human tissue during spread of the disease within the body are to a large extent initiated by these bacterial protein substances to which the patient becomes "hypersensitive" early in the course of infection, long before the progression and severity of the

disease begin. That is to say, every time the germs spread to a new place they lodge in sensitized tissue, and the reaction described ensues with its attendant train of symptoms.

If this theory concerning the nature of progressive tuberculosis is correct, it is well worth while to understand the chemical character of this protein more thoroughly. It must be confessed that there seems little possibility of neutralizing the effect in the way that diphtheria and tetanus toxins are neutralized. The protein of the tubercle bacillus does not appear to be a true "toxin" in the same sense as the two poisons mentioned. In the first place, unlike them, it is quite harmless to the uninfected person or animal. Its toxic effect is manifested only on the man or animal made "hypersensitive" by the infection itself. Moreover, it does not seem to be possible to form an "antitoxin" to it, as is so easily done in the case of diphtheria and tetanus poisons. Furthermore, it is not certain that a neutralization of the effect of this protein on the patient is altogether desirable, for the reaction itself, even though accompanied by a certain amount of tissue destruction, if not too violent, is protective.

In view of the delicate balance between the protective and the destructive effect of this "tuberculin reaction," which, as indicated, is essentially a part of the disease in its course of progression, a thorough understanding of the protein of the germ, the fraction really responsible for the reaction, is most desirable. As a matter of fact, well grounded and costly chemical studies on the nature of tubercle bacillus protein and its derivatives are well under way. Just what will come out of these studies in

practical therapy it is impossible to predict. One thing certain is that we shall cease to be in the dark as to an important agent in the manifestations of the disease.

CHAPTER X

A Hope of Mankind—Chemotherapy*

CARL VOEGTLIN, PH.D.

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Professor Voegtlin, a Ph.D. of Freiburg in Germany, started his career in this country at the University of Wisconsin (1904) and after a long sojourn (1904-13) in the Johns Hopkins Medical School was appointed Professor of Pharmacology of the U. S. Public Health Service. His investigations have covered a number of important fields, ranging from tetany to pellagra. In very recent years Dr. Voegtlin has carried out fundamental work on the scientific foundations of chemotherapy. The informed reader will recognize his work presented impersonally and modestly in the latter parts of the present article. Dr. Voegtlin represented this country in the First and Second International Conferences on Biological Standards.

THE articles in the preceding chapter dealing with "the war on invading germs" have dealt with some very important measures aiming at the control and treatment of infectious diseases. It has fallen to my lot to briefly survey the actual accomplishments and the very promising possibilities of a warfare, which only in recent years has been developed in a systematic manner. Chemotherapy—the word coined for this science by its founder and most distinguished exponent, Paul Ehrlich—aims at the destruction of invading germs through the administration of chemicals, which, while being sufficiently injurious to the germs must also be relatively harmless

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to the organs of the patient. The discovery of such chemicals, strange as it may seem to the layman, is one of the most difficult tasks confronting the medical research worker, a task which taxes to the utmost his ingenuity and patience. And this for the simple reason that all chemicals are more or less injurious to the animal body. It is indeed a matter of great surprise that many centuries ago the native Indians of Peru should have discovered the value of cinchona bark (containing quinine as we now know it) for the treatment and prevention of malaria. No less significant is the discovery of ipecac (containing emetine) by the natives of Brazil as a remedy for amebic dysentery. The discovery in the middle ages of the value of mercury for the treatment of syphilis is another example of this sort. These three important remedies, which even today are still extensively used in the prevention or treatment of these diseases, were discovered by more or less intuitive efforts to utilize vegetable and mineral products for the cure of disease. But the South American Indians had no idea how cinchona bark controlled the "fever," as the cause of the fever was then unknown. It was only in the latter part of the last and the beginning of the present century that science discovered the causative organisms of these and other diseases, and this new knowledge together with the experimental production at will of these diseases in laboratory animals furnished the basis of an experimental attack on the subject. At about the same time the rapid development of organic chemistry had furnished chemical methods for the isolation and identification of the active principles of crude vegetable drugs and thus the discovery of quinine in cinchona bark and emetine in ipecac was accomplished.

The amazing growth of the aniline dye industry in Europe, based as it was on the development of organic chemistry, supplied the medical research worker with thousands of organic chemicals for chemotherapeutic research. It was Ehrlich, a man of great vision, who for the benefit of mankind grasped the opportunities offered by the great German dye works. With the assistance of some able collaborating chemists and bacteriologists he devoted his entire energy for several years to the study of the influence of various organic dyes on the course of a disease in mice which is caused by trypanosomes. These organisms are responsible for the deadly and devastating African sleeping sickness in man and similar diseases in domestic animals. After many failures Ehrlich eventually succeeded in finding several dyes which, when injected into mice carrying the disease, produced a cure. Some of these dyes, as "Trypan blue," were found to be of practical value in the treatment of piroplasmosis. These diseases affect dogs and cattle, and produce the well known Texas fever of cattle. On the other hand these dyes were rather disappointing in the treatment of African sleeping sickness.

This work with organic dyes nevertheless laid the foundation for more important studies with the organic arsenic preparations. In 1903 the great French scientist, Lavaran, discoverer of the malarial germ, announced that by means of an injection of arsenite (Fowler's solution) he had driven out of the blood the trypanosomes of mice and that he had prolonged the life of such animals for a certain time. Soon after, an Englishman, Thomas, found that atoxyl (an organic arsenic preparation) was a much more effective remedy for this disease. This discovery led to an expedition to

tropical Africa for the purpose of trying out this new remedy on African sleeping sickness. This expedition was headed by Robert Koch—the discoverer of the tubercle bacillus—who soon after his epoch-making discoveries of disease-producing bacteria had taken an active interest in chemotherapy. The report of this expedition was of great value in demonstrating that an organic arsenical was of some use in the treatment of this dreadful disease. But unfortunately the drug had a very serious drawback in producing in some of the patients disturbances of vision and even blindness.

Invention of Arsphenamine.—Stimulated by the demonstrated efficacy of atoxyl in sleeping sickness, Ehrlich, Uhlenhut and their collaborators attacked the problem of the treatment of syphilis and related diseases by organic arsenicals. The germ responsible for syphilis had been discovered a few years previously, but all attempts at producing a curative serum had ended in failure. With a well justified healthy optimism Ehrlich and his collaborators now devoted their energy to the solution of this problem, beginning first with a thorough study of the action of various new organic arsenic preparations in trypanosome infected mice. In this investigation Ehrlich enjoyed the assistance of one of the great dye works. Through a long and tedious process of elimination more and more effective arsenicals were discovered until the 606th compound appeared to be endowed with the proper qualities of an efficient remedy. A few additional experiments were then made on the treatment of syphilitic rabbits with this chemical, which indicated such startling effectiveness in killing off the germs and in healing the lesions, that the way was paved for a trial on patients afflicted with the disease. Cautiously carried

out tests on syphilitic patients fully confirmed the results obtained with animals, and in 1909 salvarsan, as this new drug was called, was given to the medical profession. This epoch-making discovery has made possible the effective treatment of a disease which for centuries has been a curse of mankind. Salvarsan, or arsphenamine, as it is officially called in this country, when properly used in early cases of syphilis will produce a cure, with striking rapidity in the disappearance of all symptoms and signs of the disease. Patients in a more advanced stage are more difficult to cure and Ehrlich's hope of a complete eradication of the disease by a few treatments unfortunately has not materialized. As compared with the much less effective mercury, arsphenamine in a few doses will influence the disease sufficiently so that the patient cannot spread it to others. This means a great step forward in the control of syphilis, as shown by recent statistics which indicate a decided decrease in the prevalence of syphilis in certain European countries, and this in spite of the demoralizing effect of the World War.

It is evident then that arsphenamine has furnished a powerful weapon for the control of syphilis. But the research worker has not been content with this achievement, and efforts have been made to improve the drug and to find more effective substitutes. A few years ago a modified arsphenamine—sulpharsphenamine—was prepared and carefully studied at the U. S. Hygienic Laboratory as to its action in animals. This drug has certain practical advantages over arsphenamine inasmuch as it is suitable for hypodermic and intramuscular injections, whereas arsphenamine, due to its irritating properties, must be injected into the veins. Sulpharsphen-

amine, therefore, has found a place in the treatment of syphilis in infants and fat patients in which intravenous injections present great technical difficulties. Sulpharsphenamine is also superior to arsphenamine in the treatment of syphilis of the nervous system.

Another valuable contribution to this field, also made in the United States, is the invention of a drug known as tryparsamide. This arsenic preparation was first made and studied at the Rockefeller Institute for Medical Research. Its usefulness in the treatment of African sleeping sickness was established by a member of that Institute during an expedition to the Belgian Congo. A little later Loevenhart and Lorenz of the University of Wisconsin discovered the great value of tryparsamide in the treatment of general paralysis (paresis). Previous to this time this disease had resisted all attempts at successful treatment by arsphenamine. Observations now covering several years have shown that tryparsamide treatment will restore a large percentage of hopelessly insane patients to a fair degree of health, so that they can be discharged from the asylum and can resume a useful occupation.

Another important advance was the discovery of the value of bismuth in treating syphilis. Bismuth had been in use in medicine, especially in X-ray work, for many years, but it remained for the workers at the Pasteur Institute in Paris to discover that this metal is an effective antisyphilitic remedy, greatly surpassing mercury. Various drugs containing bismuth are now widely used in combination with arsphenamine.

It is a fortunate coincidence that arsphenamine is not only of value in syphilis, but also in some other diseases which are caused by closely related parasites. In tropical

and sub-tropical countries, including the West Indies and the Philippines, there occurs a disease called "yaws," which has many of the characteristics of syphilis. A large number of natives suffer from it. Up to the time of the discovery of arsphenamine the treatment of yaws was hopeless. Now we can eradicate the disease by a few injections of arsphenamine. In fact the treatment of yaws is one of the most effective treatments known for any disease. Arsphenamine is of equal value in relapsing fever, an acute disease with a high mortality, occurring in various countries.

Antimony Compounds.—Another group of drugs which has been found effective in the treatment of certain parasitic diseases contains antimony, a chemical element closely related to arsenic and bismuth. Tartar emetic, an antimony compound of tartaric acid, exerts a marked beneficial action on diseases caused by trypanosomes. The drug also is a specific for the treatment of a tropical disease called leishmaniasis or Oriental sore.

Bayer 205.—The German dye industry has always taken an active interest in the development of new medicinals. At the time of the outbreak of the World War one of the German firms took out several patents covering a series of complicated organic substances, some of which were believed to be remedies for syphilis and trypanosome infections. Soon after the conclusion of the war this firm announced the discovery of "Bayer 205" or "Germanin"; the composition of this substance was kept a trade secret. Experimental work in various laboratories showed that the drug was endowed with some remarkable properties. Animals infected with trypanosomes could be cured with a relatively small dose and it was very astonishing that such animals were resistant

for several weeks to reinfection with trypanosomes; they were temporarily immune. Great hopes were therefore placed on this drug for the control of African sleeping sickness, but the results of actual tests in Africa were somewhat disappointing. Patients in the early stages of infection respond satisfactorily but in the advanced stage, when the germs have invaded the central nervous system and have caused the characteristic tendency of the patient to sleep continuously, the drug is of no avail. It was furthermore found that Bayer 205 has to be used cautiously as otherwise it will produce kidney disease. In spite of these defects, however, the discovery of this drug may be regarded as one of the milestones in the progress of chemotherapy.

Quinine and Plasmochin.—Malaria is a disease which is very prevalent in sub-tropical countries. Large sections of the southern United States are covered with swamps where the mosquitoes, which harbor and transmit the malarial germ to human beings, can breed. There are three measures which will control malaria: first—eradication of the mosquitoes by draining the swamps or by spreading on the water surface chemicals which will kill off the mosquito larvae; second—protection of persons from being bitten by infected mosquitoes, which can be done by proper screening of houses; and third—the continuous administration of quinine to persons living in infected areas. A great deal has been written as to the relative merits of these three methods of control: suffice it to say here that the first two measures, while quite effective if properly carried out, involve a large expenditure of money which is not always available. As to the efficacy of quinine, there can be no question that it is a drug which is of great value in the prevention and

treatment of certain forms of malaria. But unfortunately quinine is of very little use in so-called malignant malaria.

In the summer of 1926 one of the large dye factories of Germany announced the invention of a new malarial remedy—plasmochin. It is claimed that this drug is effective in the treatment of all the different types of malaria, including the malignant form. It would be premature to accept this claim without further extensive corroboration of the encouraging preliminary results. Nevertheless the discovery of an artificially made drug of this kind represents a great step forward, as it opens up a field in which the chemist in collaboration with the pharmacologist and physician can search for more effective specifics, and the time will undoubtedly come when malaria will be much more successfully controlled by drugs than was possible heretofore.

DISEASES CAUSED BY BACTERIA

There can be no question that chemotherapy in the past has seen its greatest triumph in the fight against diseases caused by parasites which are classified among animal organisms (protozoa). Most of the infectious diseases of man, however, are due to bacteria, which may perhaps be regarded as lower forms of plants. At all events it is of the utmost importance to discover specifics for these bacterial diseases.

Chaulmoogra Oil in Leprosy.—The history of leprosy can be traced back almost as far as the beginning of civilization. The unfortunate individuals who have fallen prey to this dreaded disease have always been treated as social outcasts, and until a few years ago the only available means of controlling leprosy was to seg-

regate lepers in colonies. No hope being held out to them, they were left to their fate, to die of an intercurrent disease. It was known for some time that chaulmoogra oil seemed to have a favorable influence on the course of leprosy. This oil is obtained from the seeds of certain trees growing in the tropics. The crude oil has a very disagreeable taste and cannot be tolerated in large doses when given by mouth and is too irritant for injection into the muscles. In 1906 Power and his coworkers isolated from the crude oil two pure fatty acids, which were easily combined with alcohols to form esters. Later it was shown by Heiser in the Philippines and by workers at the leper colony in Hawaii that a large number of lepers respond very well indeed to repeated injections of chaulmoogra esters. The germs disappear from the lesions and the latter heal. This treatment has been found so efficient that a large number of patients have been dismissed from the leper colonies. In fact, the late Governor of the Philippines, General Wood, before his death, launched a campaign for securing a few million dollars in order that all cases of leprosy in the Islands can be systematically treated in order to eradicate the disease. At the same time work is being done in American laboratories with a view of finding even more effective remedies for this purpose.

Remedies Against Tuberculosis.—Great progress has been made in the last few decades in reducing tuberculosis. This has been largely accomplished through a systematic educational campaign as to the value of proper hygienic measures: good food, plenty of fresh air, pasteurization of milk, etc. But while the incidence of tuberculosis has been greatly cut down it is very doubtful indeed whether the time will ever come when hygienic

measures alone will completely eliminate this disease. For this reason efforts have been made to find a remedy against tuberculosis. It is possible that the future may bring the discovery of a specific serum or vaccine, but so far all attempts in this direction have been very disappointing. On the other hand, the search for chemotherapeutic agents has gone forward ever since Robert Koch's initial attempts. It will be remembered that he found that certain compounds of gold, in very high dilution, inhibit the growth of tubercle bacilli in the test tube. Work with tuberculous guinea pigs, however, showed that these gold compounds, given in doses which could be tolerated by the animals, did not exert a favorable influence upon the disease. In spite of these negative results, a few isolated workers have continued investigation along this line and only a few years ago sanocrysin, a gold compound, was hailed in the daily press as the long anticipated cure. However, carefully conducted experiments on selected patients showed that the claims made for sanocrysin could not be confirmed, and that the drug, on account of its toxic action, was altogether too dangerous for ordinary use. As far as concerns the experimental evidence on the efficacy of sanocrysin in animal tuberculosis it must be admitted that the results are very conflicting, in showing on the one hand a favorable curative action and on the other no action at all.

These remarks apply to tuberculous infection of the internal organs. In tuberculosis of the skin, so-called lupus, a large number of physicians have claimed to have observed a favorable action as a consequence of treatment with certain gold compounds. In view of the fact that this type of tuberculosis persists for many years

and resists other treatment except perhaps that with X-rays, it is at least possible that by persistent efforts specific drugs against tuberculosis may be discovered. It is very likely that if but half of the energy and money which has been spent in the search for a serum, had been devoted to chemotherapeutic investigation, greater progress might have been recorded. The difficulty is that the majority of medical men interested in tuberculosis research have been more interested in the development of serums and vaccines than in chemotherapy because their chemical training, as a rule, has been inadequate for a thorough-going chemotherapeutic investigation. There are few Paul Ehrlichs in medicine.

Remedies Against Pneumonia.—As pneumonia is an acute disease, a crisis is reached within a few days which either kills the patient or leads to recovery. In the latter case the body, through an adequate defense mechanism, can win the battle against the invading germ. In view of this fact the search for chemotherapeutic agents might possibly lead to the discovery of a chemical which would sufficiently assist the natural defense to enable the patients to overcome the disease. The difficulty is that pneumonia may be caused by several different germs. However, a promising beginning has been made, for Morgenroth has found that a chemical (optochin), closely related to quinine, is very effective in saving the life of mice infected with pneumococcus bacilli. It was disappointing, however, to find that patients could not tolerate a sufficient dose of the drug to kill off the germs due to its tendency to cause a toxic action on the optic nerves. Negative clinical results were also obtained with an organic dye, proflavine, which had shown some therapeutic action in animal experi-

ments. Quite recently some American physicians have reported promising results in the treatment of pneumonia with mercurochrome, a mercury compound invented at Johns Hopkins University. It is claimed that the patient's temperature drops several degrees soon after the injection of the drug even during the first days of the disease, and that some of the cases have rapidly recovered. Naturally these observations will have to be confirmed on a much larger scale before a definite conclusion as to the efficacy of this drug can be reached. To sum up, it can be said that while no effective remedy against pneumonia in man has been discovered as yet, experiments on animals at least hold out some promise in this direction.

Urinary Antiseptics.—In recent years there has been accumulated a large body of evidence indicating that a number of drugs when injected into the blood stream will impart to the urine antiseptic properties, and this to such an extent that the ordinary disease-producing germs of the urinary tract are killed off. To this class of drugs belong proflavine, mercurochrome, hexylresorcinol. It appears that these substances are of value in the treatment of certain infections of the urinary tract. They may be useful also for preparing patients for genito-urinary operations, which often lead to infections in spite of a most careful operative technic.

Disinfection of Wounds.—The proper treatment of extensive wounds is an important problem which confronts the surgeon. At no time was this problem more important than during the World War when thousands of soldiers came back from the firing line in a horribly mangled condition with infected wounds. The previously employed surgical procedures were found want-

ing. The credit belongs to Dakin and Carrel for having developed antiseptic solutions which could be used with impunity in treating such cases successfully. Irrigation with properly made solutions of hypochlorite of sodium (bleaching powder) and particularly of milder organic derivatives of hypochlorous acid, which readily liberate chlorine, were found to exert a very beneficial action on the healing of wounds, and undoubtedly saved many patients who otherwise would have fallen prey to a generalized infection.

Scientific Aspects of Chemotherapy.—This brief review of the actual accomplishments of chemotherapy during the last fifteen or twenty years is a sufficient demonstration of the great practical value of this new science. At the present time the discovery of new chemotherapeutic agents is still more or less a matter of good luck, for the reason that the mechanism of the action of these chemicals on the animal body and the germs is still incompletely understood. Yet it is just the clearing up of this obviously complicated mechanism which will lay a proper scientific foundation for future work. I never fail to marvel at the effect of an intravenous injection of a highly active arsenical (arsenoxide) into rats which are almost moribund from trypanosomiasis. A drop of the blood under the microscope may show more germs than red blood cells, and yet some 15 to 30 minutes after the drug injection there cannot be found a single trypanosome in the blood, and the animal to all appearance is restored to a normal condition. A simple calculation shows that five billion parasites disappear in less than half an hour from the blood of a rat weighing 100 grams (about 3 ounces) as a result of the injection of 0.13 milligram of the

arsenical! This amount of drug represents a few tiny specks. How can this phenomenal result be explained?

It was Ehrlich again who initiated work on this problem. To him it appeared of the greatest importance to find a reasonable explanation why and how arsenic kills the parasite and not at the same time the animal. Throughout his life he endeavored to explain the action of poisons on living things on a chemical basis, by assuming that the poison combined chemically with some part of the living cell. This speculation had much in its favor, but it could only be proven by actual experimentation. A long series of experiments carried out in recent years at the U. S. Hygienic Laboratory in Washington has furnished evidence to the effect that arsenic enters into combination with a sulphur compound (glutathione) which is widely distributed in animal cells and even in such low forms of plants as yeast. This sulphur compound had recently been discovered by the English biochemist, Hopkins, who found it essential for the maintenance of normal cellular combustion. Now it was significant that certain sulphur compounds have a great affinity for arsenic, as every chemist knows. It was therefore very important to find that the toxic action of arsenic on the trypanosomes could be completely checked in the test tube as well as in the living animal by simply furnishing the cells with an extra supply of glutathione. The effect of the latter substance is quite specific, as it proved to be the only substance of a great number of others occurring in the body, which afforded protection. Without going into further details we may therefore assume that arsenic kills by interfering with the normal cellular combustion or oxidation mechanism and the cells die of "internal

asphyxia." Thus it is seen that a good beginning has been made in unraveling the complex nature of the curative action of arsenic, but it will require a vast amount of painstaking work to completely clear up this fundamental problem. Progress will naturally be slow, as the action of these drugs is intimately connected with a thorough understanding of the chemistry and physics of living cells. Much less is known as to the mechanism of action of the other chemotherapeutic agents, and intensive work on these fundamental problems, including the chemistry of micro-organisms, is badly needed in order to build up a scientific basis for chemotherapy. There can be no question, however, that such knowledge ultimately would lead to practical results and the discovery of more useful drugs.

Economic Aspects of Chemotherapy.—The control or cure of any disease, no matter by what means, apart from its humanitarian effect, always represents an economic saving.

Chemistry Called Upon to Save Africa.—Among the many examples which might be used to illustrate the close relationship between chemotherapy and economics, there is perhaps none more striking than the discovery of efficient drugs for the prevention and cure of African sleeping sickness. This important disease is widely prevalent in Central and West Africa and covers about one-fourth to one-third of the area of that continent. As has been previously mentioned, the disease is caused by trypanosomes. When the so-called tetse flies bite patients suffering from sleeping sickness the flies become infected and then can transmit the disease to other human beings. This is the manner in which the disease is spread. Now it is obvious that theoretically it should be pos-

sible to eradicate the disease either by killing off all the flies or preventing their infection through biting patients. Killing off the flies in tropical Africa is almost as difficult as getting rid of the ordinary housefly in the United States, and effective isolation of every single patient suffering from sleeping sickness under present conditions is an equally hopeless task. The result is that the economic development of tropical Africa and its colonization by the white man is practically impossible. It is for this reason that Great Britain, France and Belgium, countries which own that part of Africa, are very much concerned in the discovery of effective drugs against sleeping sickness. The English institutes devoted to tropical medicine and the Pasteur Institute of France therefore have been engaged in research dealing with the discovery of effective drugs. Until recently atoxyl and tartar emetic were the only drugs possessing a marked action upon the disease, and the former is still widely used for the systematic treatment of all cases. But, as was pointed out in the introductory remarks, atoxyl is of no avail in the treatment of the later stages of the disease. The more recently developed products—tryparsamide and Bayer 205—are superior to atoxyl. All these remedies must be injected into the veins, and this obviously restricts their use. Experiments are therefore in progress to substitute these drugs by some which can be given by mouth, as quinine in malaria. Should these experiments be crowned with success, there could be little doubt that the whole economic situation of tropical Africa would be very much improved. The natives would be protected from this dreaded disease which carries off thousands each year, and the white

man would no longer be obliged to risk his life in these countries.

Yaws and malaria also occur in tropical Africa. The former disease, as we have seen, can be easily controlled by arsphenamine or bismuth, and quinine is effective in the prevention of malaria. But here also the future holds out hope for the discovery of better remedies, and the time will come when the vast natural resources of fertile tropical Africa may be made available for the benefit of mankind.

In a similar way the jungles of tropical America will yield an enormous territory to the advancing civilization, and there is no doubt that chemotherapy will play an important part in this development. Our own country would also be enormously benefited by effective remedies against tuberculosis, pneumonia and similar diseases which each year carry off thousands of persons in the prime of their life.

It would be a mistake, however, to expect chemotherapy to accomplish more than can be reasonably expected of it in its present development. Thus, it would be absurd to anticipate the discovery of a drug which would cure every single patient. After all, the use of serum in diphtheria does not cure all cases, but its use has greatly lowered the death rate, and no intelligent person would therefore minimize the benefits derived from serum treatment. It is in the nature of chemotherapy also that the patient will run a slight risk of being affected by the toxic action of the drug. But if one considers, for example, the millions of patients who have received arsenical treatment during the last 17 years it is perfectly obvious that the number of serious reactions and toxic deaths have been insignificant.

How Are Valuable Specifics Discovered?—There was a time—and this is still true to a limited extent today—when great discoveries in medicine were made by investigators of vision working quietly ahead in simply equipped laboratories. That is how Pasteur and Koch made some of their early epoch-making discoveries. But it would have been utterly impossible for Ehrlich to discover a specific against syphilis under such primitive conditions. In fact, Ehrlich, in announcing the discovery of salvarsan, did not hesitate to give full credit for the financial help which allowed him to organize a splendid institute for chemotherapy. It was there that he gathered around him a group of excellent chemists, who in cooperation with a large dye firm prepared the hundreds of different arsenicals, which were tested out carefully on animals by a competent staff of biologists. The expense involved in the preparation of these new chemicals was very considerable, and, of course, the same was true in carrying out the thousands of animal experiments. But Ehrlich, as he frankly admits, was not parsimonious in the expenditure of money, for he realized that only an investigation on a large scale could be crowned by success. The policy of his institute, founded in 1906, has not changed to the present day. It is still by far the most active and well equipped laboratory in the world for chemotherapeutic research and it still has close connections with the same great dye works. It goes without saying that valuable discoveries have been made in laboratories which were not organized for the specific purpose of chemotherapy, but these institutions, as a rule, are not properly organized with regard to personnel and physical equipment, and very often work is prematurely discontinued, due to lack of financial sup-

port. In some cases work was stopped because the director of the institution had not sufficient patience to await practical results, which often is a matter of years. In this respect we could profit by the lessons of the German method. With the overabundance of wealth in this country it should indeed be possible to organize a laboratory of the type of Ehrlich's institute with a staff of excellent chemists, pharmacologists, bacteriologists and any other specialists who would devote their entire energy to a cooperative attack on chemotherapeutic problems. Such an institute should have the active support of the organic chemical industry. Since the war the latter industry in our own country has grown to enormous proportions, but it is rather disappointing to find that it does not encourage fundamental research, being far more interested in the exploitation of already existing knowledge. The secret of the success of the immense organic chemical industry in Germany is to a great extent due to their policy of establishing within the works true research laboratories which, as far as personnel and equipment are concerned, are not surpassed by the chemical departments of German universities. These factories in addition maintain a large staff of consulting chemists who rank among the most distinguished chemists, such men as Baeyer and Emil Fischer. I hope that the day will come when our organic chemical industry will follow the example of the General Electric Company in this respect. At all events I hope that the time is not too distant when the United States will contribute to the world new chemotherapeutic agents which will rank in importance with Ehrlich's salvarsan. If nothing else national pride should be a sufficient incentive.

GLOSSARY

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ACIDOSIS
Condition of excessive acidity in the body, caused by excess formation of acid or by depletion of base.

ACROMEGALY
A disease of man, manifested by giantism, due to a tumor of the pituitary gland with increased secretion therefrom.

ALBINISM
A lack of pigment formation indicated by very light hair, pink eyes, a very pale skin.

ALIMENTARY
Pertaining to food, or to the digestive apparatus of the body.

ALVEOLI
Small cells or cavities lined with functioning cells.

AMPHIBIA
Animals which can live both on land and in water.

AMPOULE
A small glass vial.

ANAEROBIC
Referring to bacteria which thrive in the absence of air (oxygen) and will not grow in the presence of oxygen.

ANAPHYLACTIC
Pertaining to the increased susceptibility to a foreign protein following a first absorption of the same protein.

ANCHYLOSED JOINTS
Bony stiffening of joints, with bone growth.

ANEMIA
Deficiency of the hemoglobin of the blood.

ANTHELMINTIC
Referring to the property of expelling intestinal worms.

ANTINEURITIC
Pertaining to the property of protecting the nervous system and its parts, as an antineuritic vitamin.

ANTISCORBUTIC
Relating to the property of preventing scurvy.

ANTITOXIN
A chemical (or chemicals) produced by the living body which reacts with and alters to the point of being innocuous, a poison or toxin.

ARTERIOLE
A small artery.

ARTERIOSCLEROSIS
Scarring or hardening of arteries as a result of disease in the arterial walls.

ASEPSIS
Condition of freedom from bacterial contamination. Absence of sepsis.

ATONIC
Lack of tone.

ATRETIC (or atresic)
Without a usual opening; imperforate.

ATROPHY
Shrinkage and wasting of tissues.

AUTOCATALYTIC
The property of a chemical substance, which renders it capable of accelerating its own transformation.

AUTOLYSIS
Autodigestion.

BACTERIOLOGY
The science that studies bacteria and other micro-organisms.

BACTERIOPHAGE
Unknown form of life or substance which destroys bacteria.

BIOCHEMISTRY
A branch of chemistry which studies the chemistry of living matter.

BRACHYDACTYLY
A characteristic lack of the full complement of joints in the fingers or toes; possessing short fingers.

CALCIFICATION
Deposition of lime salts in the tissues.

CALORIE
A unit of heat used frequently to express the fuel value of food. The calorie ("large calorie") is the heat required to increase the temperature of 1000 grams of water one degree (from 0° to 1°, centigrade scale).

CARBOHYDRATE
A class of foods and related products including glucose, cane sugar, starch, cellulose, etc., containing carbon combined with hydrogen and oxygen only. In the best known of these, hydrogen and oxygen are in the ratio of two atoms of hydrogen to every atom of oxygen, as in water, H_2O. Hence the name carbo*hydrate*. Glucose is $C_6H_{12}O_6$.

CARCINOMATOUS
Relating to malignant cancer of epithelial cells.

CARDIAC
Relating to the heart.

CAROTID
The chief arteries of the head and neck, one on either side.

CATHETER
A hollow, flexible tube, used to drain fluid, as from the bladder.

CELL
The unit of life; a minute mass of protoplasm with a nucleus, with or without a limiting wall, which has the power of producing other cells and of carrying on other vital processes, such as motion, digestion, secretion, etc.

CENTRIFUGE
A device for separating parts by a rapid rotary motion (e.g., cream from milk), which depends on the centrifugal forces of rotary motion.

CERVICAL
Referring to the neck of any structure.

CHROMATIN
A substance in the cell nucleus which determines the nature of daughter-cells; the chemical carrier of inheritance in any cell. It is readily stained by dyes (hence the name).

CHROMOSOME
Unit of chromatin in the cell nucleus.

CLEFT
A fissure or abnormal opening.

COALESCENCE
A growing-together or union.

COLLOID
A glue-like or gelatinous substance. COLLOIDAL SUSPENSION: A mixture in which

particles are held in suspension, such as drops of fat in milk, gas bubbles in foam, etc. The forms and surfaces of the particles change easily with the forces acting on them and are not rigid as in crystals.

CONJUNCTIVITIS
An inflammation of the mucous membrane of the eyelids.

CORONARY VESSELS
Blood vessels of the heart, supplying blood to all parts of the wall.

CORTEX
The outer portion of a gland or structure; the rind.

CYSTINE
An amino-acid which forms one of the components of proteins and contains sulphur.

CYTOLOGY
The science which studies the structure and functions of cells.

CYTOPLASM
The protoplasm or living substance of a cell outside of its nucleus.

DESQUAMATE
To scale off or peel off (as in the scaling off of the skin in scarlet fever).

DIMORPHISM
Occurrence in two different forms. Applies to parts of a plant, such as leaves or flowers, to parts of an animal, or to chemical substances. Thus, crystallized carbon occurs as diamond and as graphite.

DIPHTHERIA
A specific infection with the diphtheria bacilli, characterized by marked general poisoning (intoxication) by toxins produced by the bacteria.

DIURESIS
Rapid secretion of urine; stimulation of formation of urine.

ECTOMY
A word-ending which refers to the excision of a part, as in appendectomy, the excision of the appendix.

EFFLUENT
Flowing out.

ELECTRIC POTENTIAL
The electric equivalent of the "head" or difference in level of source and outlet of water in a reservoir. The work that can be done by the water is proportional to the product of the "head" and the quantity of water. The work that can be done by electricity is likewise proportional to the difference in electric potential (this is measured in *volts*) between two points and the quantity of electricity available.

EMBRYO
Early stage of development of an organism, before it is fully formed: as a chick within the egg.

ENDEMIC
Habitual, established incidence of disease in a locality.

ENDODERM
The innermost layer of cells, e.g., of an embryo.

ENUCLEATE
To remove the nucleus or center.

ENZYME
An organic substance, produced in living cells, that accelerates chemical action. Thus,

pepsin, produced by the lining of the stomach, accelerates the digestion (hydrolysis) of proteins.

EPIDEMIC
A widely prevalent disease, as an epidemic of cholera.

EPIPHYSES
The end portion of long bones, which develops separately from the shaft. Epiphysis cerebi: The pineal gland.

EPITHELIUM
A covering, surface layer of cells.

ETIOLOGY
The science of causes, as of a disease.

EUGENICS
The science of improving the human race through its offspring.

EUNUCH
An unsexed (castrated) man.

EXACERBATION
An increase or aggravation of a process, as of fever, rapid pulse, anemia or pain.

EXOPHTHALMIC
Associated with protruding eyeballs.

FEMORAL
Relating to the femur or the long bone of the thigh, or to the region of the femur.

FERTILIZATION
The union of a male element (e.g., a spermatozoon in animals, pollen in plants) with the female reproductive cell (the egg or ovum in animals, the ovule in plants), which starts the development of a new individual.

FETAL
Pertaining to the fetus, the young of an animal while still in the womb.

FOLLICLE
A small sac or cyst containing some substance, as for example the hair follicle.

GENE
The factor or differential substance in a germ cell (or other cell) that determines a given character.

GENETICS
Genetics is the science which seeks to account for the resemblances and the differences exhibited among organisms related by descent.

GERMINAL
Pertaining to a germ or germ-cell.

GESTATION
Period of pregnancy; phenomena of pregnancy.

GLOMERULI
Small structures in the kidney composed of capillary blood vessels in a cluster and surrounded by a thin wall.

GLYCEMIA
The presence of sugar (glucose) in the blood; hyperglycemia, an excessive amount, hypoglycemia, an unusually low concentration, of sugar in the blood.

GLYCOSURIA
Abnormally great secretion of sugar in the urine.

GRAM
The unit of mass (weight) used in science. A gram is equal to 15.4 grains (apothe-

cary). One ounce (avoirdupois) is equal to 28.4 grams.

HEMOLYTIC
Pertaining to the property of dissolution of the red blood corpuscles.

HEPATIC
Relating to the liver.

HEURISTIC
Serving to find out or discover.

HISTOLOGY
The science of the minute structure and function of the tissues of the body; microscopic anatomy and physiology.

HOMOZYGOUS
Breeding true to type.

HORMONE
A chemical product manufactured by some tissue, such as a gland, which carried by the blood, acts as a messenger and controls other tissues, as by stimulation or depression. Growth, sex characteristics, effects on the heart beat are instances of such control.

HYDROGEN ION CONCENTRATION
Hydrogen ions ($H+$) are formed by acids and give acids their acid character; the concentration of these ions in the blood or other liquids has a very vital effect on all life processes.

HYDROSTATIC
Pertaining to the pressure exerted by liquids and on liquids. Medically also: stagnation of fluid.

HYPERPLASIA
Abnormal multiplication of cells; rapid growth.

HYPERSENSITIVE
Increased sensitiveness.

HYPERTROPHY
Increased size due to growth.

HYPOPHYSECTOMY
Removal of the hypophysis or pituitary gland, a small gland at the base of the brain.

HYSTERIA
A nervous disease without known pathologic basis.

INFUNDIBULAR
Pertaining to a funnel shaped passage; applied to the passage of the pituitary extending down from the brain to certain portions of the kidney, part of the nose, etc.

INGESTION
The taking in, e.g., of food.

INTERSTITIAL
Structures, such as cells, lying between other structures; a supporting framework of tissue within an organ.

INTRAMUSCULAR
Within, or into, a muscle.

INTRAVENOUS
Within, or into, a vein.

IN VITRO
In artificial conditions, resembling those of life; e.g., in a glass vessel.

IN VIVO
In life; in the body. (In contrast to *in vitro*).

KERATINIZE
To form a horn-like substance; to harden and to thicken, as said of the skin in the formation of a callus, finger-nails and horns.

KERATOMALACIA
Softening of the horny (outer) layer of the skin.

KILOGRAM
A unit of weight, equal to one thousand grams, or 2.2 pounds avoirdupois.

LABILE
Unstable, liable to change.

LACHRYMAL
Pertaining to the tears, tear glands and tear ducts.

LESION
A local abnormality; bruise, wound, scar, inflammation, cavity, etc.

LEUCOCYTES
The white cells of the circulating blood. They are the scavengers and warriors of the blood.

LEUCOCYTOSIS
Increase (abnormal) in the number of leucocytes.

LEUCOPENIA
Deficiency in the number of leucocytes.

LOCKJAW
A disease caused by the tetanus germs, in which the jaws become firmly locked because of muscle spasm.

LUTEA
A part of the ovary.

LYMPH
The fluid of the tissues, outside of the blood-vessels, having its own circulation and carrying away most of the waste from tissues, later being filtered and poured into the blood.

MACROPHAGES
Large white cells of the blood, having the power of ingesting bacteria, debris, etc.

MALNUTRITION
Defective nutrition.

MAMMAL
An animal belonging to the mammalia, the young of which are fed at the breast.

MATRIX
A mold; the intercellular substance of tissues; the womb.

METABOLISM
The sum of the chemical processes by which food is used in the body for the development of heat, the doing of work, the formation of new tissues, etc.

METAMORPHOSIS
A change of form or structure.

MIASM
Injurious exhalations or germs infecting the air.

MOLLUSK
A class of animals, such as oysters, snails, mussels, characterized by protective hard shell coverings.

MORBIDITY
Degree of sickness; prevalence of sickness.

MORIBUND
In a dying state.

MORPHOLOGY
The science of the structure of cells and tissues.

MOTILE
Capable of spontaneous motion.

MOTOR REFLEX
A reflex involving stimulation of a motor nerve; a reflex which produces motion or activity.

MUTATION
An inherited change in the germ plasm, which produces changes in the body.

MYXEDEMA
A condition of a peculiar swelling of tissues, due to thyroid deficiency and associated with low metabolism and stunted growth and mentality.

NECROSIS
Death of cells or tissues.

NEURASTHENIA
Nervous exhaustion.

NEURITIS
Inflammation of a nerve.

NEURALGIA
Pain along the course of a nerve.

NUCLEUS
The defined central part of a structure, such as the nucleus of a cell, or of an atom. The portion of a cell necessary for its life and reproduction.

OLFACTORY
Pertaining to the sense and organs of smell.

OPHTHALMIA
Severe inflammation of the eye.

ORAL
Pertaining to a mouth.

ORGANIC MATTER
Matter containing carbon; originally it was erroneously thought that such matter could be produced only by living organisms.

ORTHOSTATIC
Pertaining to the position of the body, e.g., horizontal or vertical.

OSTEOPOROSIS
Rarefaction of bone by enlargement of its cavities or formation of new spaces.

OVARIOTOMY
Opening of the ovaries.

OVOGENESIS
The origin and development of the ovum or egg.

PARATHYROIDECTOMY
Removal of the parathyroid glands; they are located adjacent to the thyroid gland.

PARESIS
A paralytic disease of the brain, a late stage of syphilis.

PARI PASSU
At an equal rate; at an equal pace.

PAROXYSM
A sudden, violent outburst.

PARTHENOGENESIS
Reproduction from eggs or ovules without the aid of fertilization by the male element (sperm, pollen).

PATHOGENESIS
The development of a disease.

PATHOLOGY
The science which studies the changes caused by disease.

PEDIATRICS
The branch of medicine dealing with the diseases of children.

PERCUTANEOUS
Through the skin.

PERICARDIUM
The membranous sac covering and surrounding the heart.

PERIPHERAL
Pertaining to the periphery, outside or boundary. The peripheral nervous system is that outside of the skull and spinal canal.

PERITONEAL
Pertaining to the membrane lining the abdominal cavity (peritoneum).

PHAGOCYTOSIS
Process of ingestion of larger particles (e.g., bacteria) by cells.

PHARMACOLOGY
The science which studies drugs and their effects.

PHOTOCHEMISTRY
The science which studies chemical changes as affected by light.

PHYSIOLOGY
The science which studies the functions of living organisms.

PIROPLASMOSIS
Infection by piroplasma, an ovoid parasite of the blood, which causes Rocky Mountain spotted fever.

PLACENTA
The structure attaching a fetus to the wall of the uterus, where food passes from the mother's blood to that of the fetus; the afterbirth.

PLANARIA
A group of flatworms.

PLASMA
The liquid part of blood or lymph.

PLASMODIUM
A micro-organism in the blood, causing malaria and similar diseases (see piroplasmosis).

PLEURA
The external lining of the lungs and chest cavity.

POLARISM
The property of possessing poles (magnetic, electric, cellular, etc.); pertaining to a pole.

POLYNEURITIS
Inflammation of more than one peripheral nerve; multiple neuritis.

PRAGMATIC
Dealing with causes and effects, with practical consequences.

PRESSOR
Stimulating; increasing tone (e.g., causing an increase in the blood pressure).

PROLIFERATION
Growth by multiplication of parts, as in cell division.

PROPHYLACTIC
Pertaining to prevention of disease.

PROTEOLYTIC
Pertaining to the breaking down of proteins, as in digestion.

PROTOSCOPICALLY
Pertaining to inspection of protoplasm.

PROTOZOA
Animal organisms consisting of single cells.

RACHITIC
Pertaining to rickets.

RENAL
Pertaining to the kidneys.

SEMINAL
Pertaining to the semen, the sperm, or male reproductive elements.

SENILITY
The infirmities of old age.

SEQUELAE
Sequels or after-effects.

SKELETAL
Pertaining to the skeleton.

SOL
A persistent suspension of minute particles in a liquid; a colloidal suspension of solid, liquid or gas particles in a liquid, as in milk, foam, etc.

SOMATIC
Pertaining to the body, as opposed to the germ plasm.

SPASTICITY
A tendency to spasm or violent contraction; increased tonus.

SPECTRUM
The band of colors produced when white light (such as sunlight) is refracted and dispersed by a prism or similar device. The colors of the rainbow represent a spectrum produced by the refraction and dispersing of sunlight by drops of rain.

SPHINCTERS
Ring-like muscles closing an orifice or passage (as in the intestine).

SPLANCHNIC NERVE
A large and important nerve in the abdomen, enervating the organs of the abdomen.

SPORADIC
Occasional, scattered.

STEREOISOMERISM
Chemical compounds which have identical composition but different properties are called *isomers*. Stereoisomerism is an isomerism which depends wholly on the arrangement in space of the atoms in the molecules of compounds which have identical composition. The relation of the right to the left hand illustrate one form of such differences in space relationship.

STEREOTROPIC
To see with both eyes; to have focussed from both points, as in stereopticon pictures.

STERILE
Unproductive.

STREPTOCOCCUS
A bacterium, round in shape, and occurring in chain-like arrangement.

STROMA
The framework of an organ or cell; the matrix.

SUBCUTANEOUS
Beneath the skin.

SUPPURATIVE
Pus producing.

SUPRASTERNAL
Above the sternum or breast bone.

SUTURE
The uniting of parts by stitches (surgery); the line of junction between adjacent parts.

SYMPTOMATOLOGY
The combination of symptoms; the symptom complex.

SYNDROME
The complete picture of a disease, including all of the symptoms.

TACTILE
By touch.

THERAPY
Treatment of disease.

TONUS
The state of continuous partial contraction of a muscle or muscles; tone; tenseness.

TOPICAL
Medically: local (as topical treatment).

TOXIC
Poisonous.

TOXICOLOGY
The science that studies poisons.

TRABECULAE
Partitions (septa) which extend from the enclosing wall or envelope into the enclosed substance.

TRAUMATIC
Pertaining to physical injury, from a blow, pressure or torsion.

TROPISM
The tendency of living organisms to move or turn in response to an external stimulus (such as a beam of light, an electric charge, etc.).

TRYPTIC
Pertaining to trypsin, a digestive ferment of the pancreatic juice.

VAGUS
The twelfth pair of nerves from the brain, controlling the heart, lungs, blood vessels and most of the abdominal organs.

VASCULAR
Relating to tubes or vessels, such as blood-vessels. The vascular system is the system of blood-vessels in the body.

VASOCONSTRICTION
Constriction of vessels, such as blood-vessels.

VERTEBRATE
An animal possessing a spinal column; relating to a vertebrate.

VIBRIO
A species of microbes.

VIRILISM
Masculinity; normal in the male, but a disease in women, characterized by changes in voice, figure, hair, etc.

VISCOSITY
The force required to overcome the resistance of a fluid or gas to changes of form.

XEROPHTHALMIA
A form of degenerative disease of the eye; atrophy of the conjunctiva.

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